CNS DRUGS

A Symposium held at the
Regional Research Laboratory
Hyderabad, India
January 24–30, 1966

COUNCIL OF SCIENTIFIC & INDUS
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CNS DRUGS

Research on CNS Drugs is multidisciplinary. Because of the specialized skills involved, each of a high order, chemists, pharmacologists and manufacturers have tended to work apart, and a common platform for discussion seemed desirable. This publication is a compilation of papers presented at an international symposium on CNS Drugs held at the Regional Research Laboratory, Hyderabad, India, from January 24 to 30, 1966, under the sponsorship of the Council of Scientific & Industrial Research, New Delhi.

The symposium was attended by 36 invited participants from all over the world, including India, and by a large number of observers from nearly every centre of activity in this field in India. The papers here presented cover diverse aspects of the subject, such as the chemistry and pharmacology of new and known drugs, structure-activity relationships, theories on mechanism of action and development of tolerance. Brought together here is an up-to-date account of world activity in this field.

The publication should be of interest to everyone who has been engaged in research on CNS Drugs from whatever point of view, and indeed to all those interested in drugs or drug action.

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DEDICATED TO

Dr S. Husain Zaheer
in appreciation of his
contribution to the initiation and
development of research in this field in India

Preface

This publication is a compilation of papers presented at a symposium on Central Nervous System (CNS) Drugs held at the Regional Research Laboratory, Hyderabad, India, from January 24 to 30, 1966, under the auspices of the Council of Scientific & Industrial Research, New Delhi. The symposium was attended by 36 invited participants from Canada, Czechoslovakia, France, Germany, Italy, the UK, the USA, Yugoslavia and India and observers from some 20 institutions in India. A total of 32 papers were presented and discussed.

Prompting this symposium was the obviously multidisciplinary nature of research in the area of CNS Drugs, coupled with its recent impressive growth. The relationship between chemical structure and biological activity is still not clear, nor is the mode of action of CNS drugs. Because of the specialized skills involved, each of a high order, chemists, pharmacologists and manufacturers tend to work apart. A meeting of minds could well be of importance to the further effort in this field by bringing home to each discipline the methodology of the other. Thus, the papers presented covered the following aspects of CNS Drugs: (i) chemistry and pharmacology of new drugs and known drugs, (ii) structure-activity relationships, and (iii) theories on the mechanism of action, development of tolerance and related subjects.

The success of the symposium was due in large measure to the inspiring guidance and continued support given it by Dr S. Husain Zaheer in his capacity as Director-General, Council of Scientific & Industrial Research, New Delhi. Dr Zaheer had earlier initiated research in medicinal chemistry in this country first in the Chemistry Department of Lucknow University and from about 1949 at the Regional Research Laboratory in Hyderabad, as its Director. This volume is dedicated to Dr Zaheer in appreciation of his interest in this field of research in particular and for his support of the international symposium from which this publication has arisen.

We take this opportunity to thank Dr E. Schlittler, Dr Nitya Anand, Dr E. L. May, Dr R. S. Grewal, Dr M. Protiva, Dr U. K. Sheth, Dr J. R. Boissier, Dr H. L. Borison, Prof. G. Fodor and Dr P. B. Bradley for presiding over the various sessions and conducting the proceedings of the symposium. To Dr May and Dr Borison we are also particularly grateful

for two popular evening lectures on "Analgesics and drug dependence" and "Body temperature regulation", given during the symposium.

We are indebted to a large number of colleagues in this laboratory for meticulous conduct of the various organizational aspects of this symposium, in particular the groups working in the areas of organic chemistry and liaison and information. We are grateful to Shri S. B. Deshaprabhu, Scientist Incharge, Publications & Information Directorate, CSIR, for bringing out this publication in the most expeditious manner.

Regional Research Laboratory
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August 15, 1966

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Structure-Activity Relationship in Rauwolfia Alkaloids

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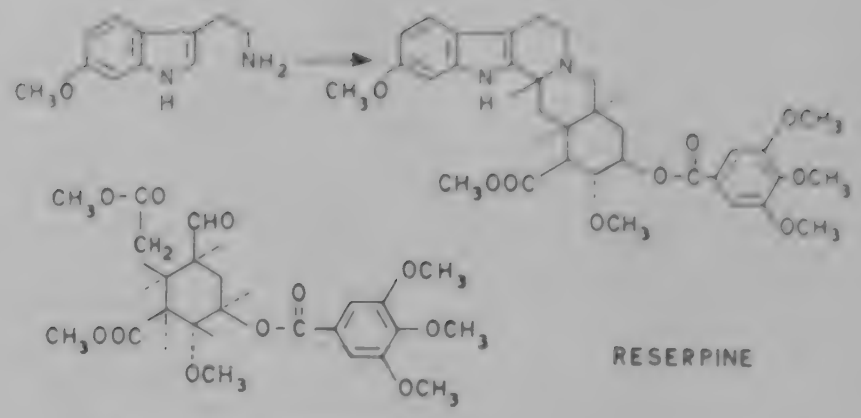
It is now about 14 years since reserpine has been isolated from *Rauwolfia serpentina*[1] and in the years following, an enormous amount of chemical, pharmacological, clinical and other work has been done and many hundreds of papers have been published in this field[2]. The discovery of reserpine has initiated a great boom in the investigation of indole alkaloids and natural products in general[3]. It should be pointed out here that reserpine was originally hailed as the tranquillizer of the century, but because of its depressive properties it lost its ground to a number of simultaneously introduced synthetics. Also its depressive activities were found to limit somewhat its use as an anti-hypertensive compound. Studies on the modification of the reserpine-molecule yielded compounds with either preferentially sedative or anti-hypertensive activity, but basically no new activities with therapeutic possibilities were found.

In 1956 Woodward published a synthesis of reserpine[4] whose principal feature was an ingenious method for the creation of the potential ring E of the alkaloid with five contiguous asymmetric carbon atoms in the proper orientation.

French[5], Swiss[6] and Czech[7] chemists developed this procedure to high technical perfection and, for a time, man-made reserpine successfully competed in price with the isolated natural product. This synthesis opened an alley to a great number of analogous compounds with modifications in rings A, B, and C which could not have been derived from the natural substance. Investment in this work was very great but, apart from a successful technical synthesis of reserpine, the results can be summarized by saying that "no change would have been for the better". This contrasts quite distinctly with the situation in the steroid field, where semisynthetic steroid derivatives have long overtaken in importance the natural hormones. In



WOODWARD SYNTHESIS

this connection I would remind you of the many derivatives of prednisone and of fluorinated prednisone.

Definition of Activities

Although we have been invited to attend a symposium on CNS-active substances, I have so far accentuated the anti-hypertensive activity of reserpine. This is perhaps the moment to say something about the nature of the activities of the compounds which I am going to discuss. Plummer, one of the pioneers in this field, is of the opinion that the mechanism of action of reserpine and its congeners is not fully known yet. Brodie[8] has postulated that the sedative and anti-hypertensive activities of reserpine are caused by the depletion of the brain and the peripheral vessels of serotonin and of catecholamines. The difference between the two activities is a manifestation of degree, i.e. for reserpine and its ethers, the brain is the site of primary attack and hence their sedative action; other reserpine derivatives (to be discussed later) act primarily upon catecholamines of the periphery (blood vessels) and, therefore, are anti-hypertensive.

For the purpose of this paper, I am going to regard manifestation of these differences as a matter of degree which is dose-dependent. Therefore, I believe it is proper for me to talk at this symposium about the anti-hypertensive effects along with the sedative.

The role of serotonin as a cause of reserpine-induced sedation is a very controversial matter. As stated in the recently published third edition of Goodman and Gilman's "Pharmacological Basis of Therapeutics", the exact role of serotonin, dopamine and norepinephrine in brain function is unknown. It is quite possible that reserpine acts *per se* or by some other unrecognized intermediary step. Depletion of brain serotonin, however, is not necessarily accompanied by sedation[9] because *dl-p*-chloro-*N*-methylamphetamine lowers serotonin levels to 35 per cent of control in rat brain without causing sedation. The converse is also true, since chlorpromazine produces a reserpine-like sedation without depleting brain amines.

The following experiments[10] contribute further negative evidence to the serotonin approach. Rats on a tryptophane-free diet had brain serotonin levels of only 25 per cent of those of control animals. When reserpine 5 mg./kg. was administered on the ninth day of the diet, sedation was produced in both the control and treated animals. After 21 days, sedation disappeared and serotonin levels returned to normal in the controls but remained low in the tryptophane-deficient animals. Another dose of 1 mg./kg. of reserpine on the twentythird day, produced sedation in both the groups of rats. There was no difference in the type of sedation or in the electroencephalographic patterns in the two groups of animals. It was, therefore, concluded that the central nervous action of reserpine does not depend on the serotonin level in the brain.

I shall not say anything more about the pharmacology of reserpine but deal with some of the problems of structure-activity relationship. This work in the reserpine field has centred around three specific areas. (a) Synthesis of model compounds with fewer or possibly no asymmetric carbon atoms. This includes a wide variety of derivatives, from compounds which still retain a resemblance to reserpine, to others which are simple derivatives of trimethoxybenzoic acid. (b) Synthetic analogs and homologs of reserpine, mostly but not always obtained by the Woodward synthesis. (c) Stereoisomers of reserpine, mostly derivatives of natural alkaloids. Group (a) has yielded no therapeutically useful compounds. A few compounds had a short-lived hypotensive activity which was probably an expression more of toxicity. Some sedative products were also obtained which, nevertheless, may have quite a different reaction mechanism. Group (a) will, therefore, not be dealt with in this presentation. Investigation of (b) and (c) has demonstrated that activity depends on the presence or absence of certain functional groups. In addition, the stereochemistry of the pentacyclic molecule is of prime importance. This then will be the main theme of my further presentation.

Synthetic Analogs and Homologs obtained mainly by Woodward Synthesis

Figure 1 summarizes some of the simple alterations in the reserpine structure and the resultant activities.

Ring A

Since deserpidine is as active as reserpine, it is evident that the methoxy group in ring A is not essential. Ring A can be brominated[11] and acetylated[12], the latter derivatives being inactive. *Via* Woodward synthesis, a large number of substituted tryptamines have been used to produce a large number of analogs. The best known analog is 10-methoxydeserpidine (Decaserpyl)[13] which is said to be exclusively hypotensive, whereas 10-chlorodeserpidine is claimed to be only sedative[14]. As I have already

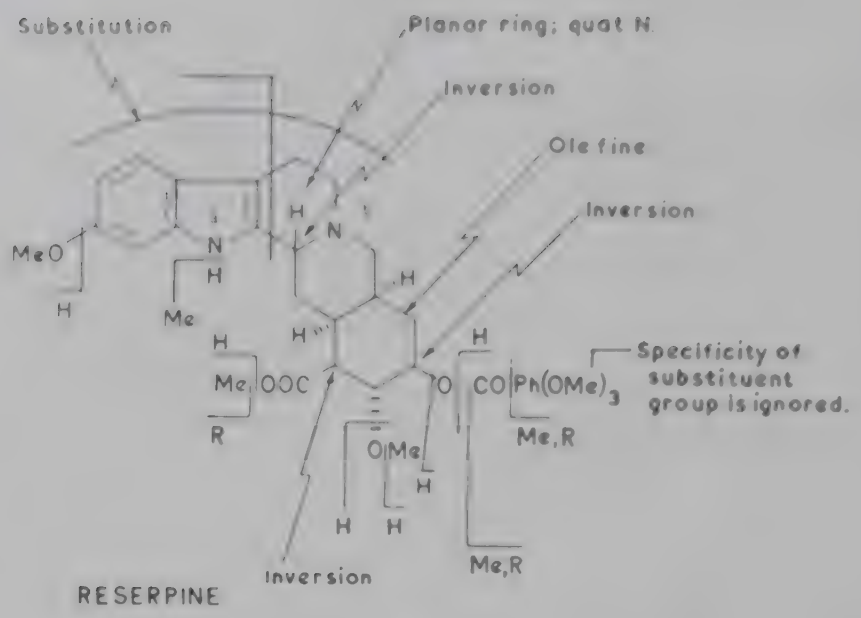


FIG. 1

mentioned, the manifestation of the response is dose-dependent and these claims are properly interpreted as expressing degrees rather than total exclusion. It is reported that 10-methoxydeserpidine is clinically also sedative at a multiple of the hypotensive dose[15], which is about 30 mg. day as compared with 0·3 mg. for reserpine.

Other reserpine analogs obtained by the Woodward synthesis have one or more alkyl, alkoxy, chloro, fluoro, hydroxy, alkyl-mercapto, etc. substituents at carbon atoms 9, 10, 11 and 12 and indications as to their activities can be found in papers of our French and Czech colleagues[16].

Ring B

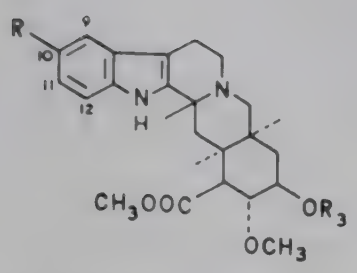
Here modifications are listed of the nitrogen N_a and it has been found that in certain cases activity is destroyed (N_a-CH_2-$CH=CH_2$ or even stimulating effects are obtained when N_a is substituted by a methyl group[17]. Czech investigators have also replaced N_a by a sulphur atom in a synthesis of 1-desaza-1-thiadeserpidine but their synthesis has only been pushed to the level of the 3-*iso* compound which we know from experience is inactive[18].

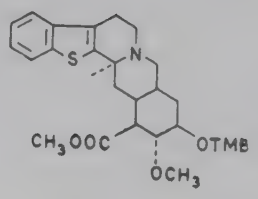
Ring C

By total synthesis compounds with alkyl groups at C-5 and C-6 have been obtained and it is claimed that these compounds retain activity[19]. Less surprising in view of the drastic change in polarity is the fact that quaternization of N_b destroys any reserpine activity[20].

Ring D

By a slight modification of the Woodward synthesis, Czech investigators





R = CH_3O - 10-methoxydeserpidine, Decaserpyl Ⓣ – hypotensive

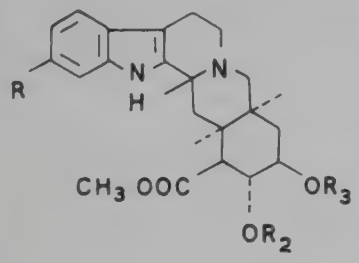
R = Cl – 10-chlorodeserpidine – sedative

R_3 = TMB in both cases.

have prepared a deserpidine analog[21] with a methyl substituent in position 19 but no definite statements as to the activity of such a compound are made.

Ring E

Nature itself has provided us with a number of compounds differing from reserpine and deserpidine in Ring E and it is appropriate to first look at these alkaloids. Rescinnamine is methyl trimethoxycinnamoyl reserpate and is widely distributed in Rauwolfia species[22]. Quantitatively it is weaker than reserpine and deserpidine, but qualitatively it has the same action. Then we have the three natural alkaloids with OH groups instead of methoxy groups in position 17 α : ψ-reserpine, rescidine and raunescine, all of which are still active, although in a reduced sense.



R = OCH_3; R_2 = CH_3; R_3 = TMC – Rescinnamine

R = OCH_3; R_2 = H; R_3 = TMB – ψ-Reserpine[25]

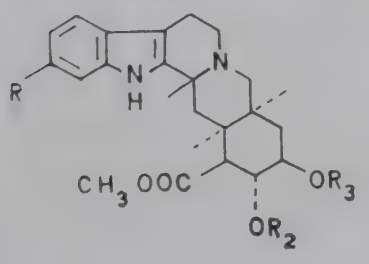
R = OCH_3; R_2 = H; R_3 = TMC – Rescidine[26]

R = H; R_2 = H; R_3 = TMB – Raunescine[27]

TMB = trimethoxybenzoyl

TMC = trimethoxycinnamoyl

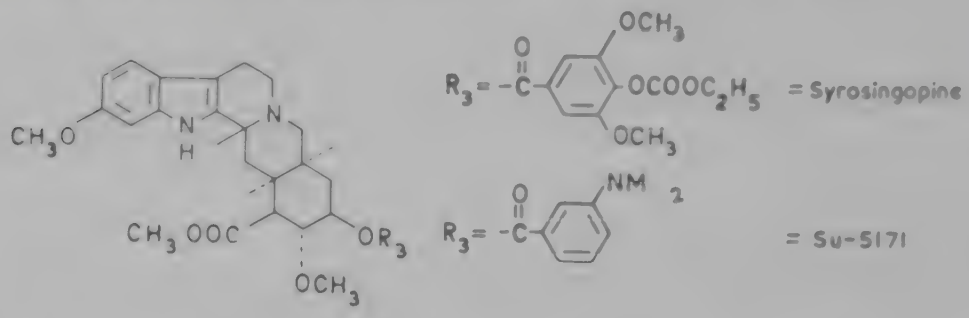
In contrast with this first group of isomers, alkaloids raugustine and isoraunescine with free hydroxyls in 18 and aroylesters in 17 are inactive. Reserpic acid, methylreserpate and reserpic acid 18-trimethoxybenzoate[27] are inactive and from this finding it was concluded that in both 16 and 18 positions reserpic acid had to carry some kind of a 'protective group'. Since



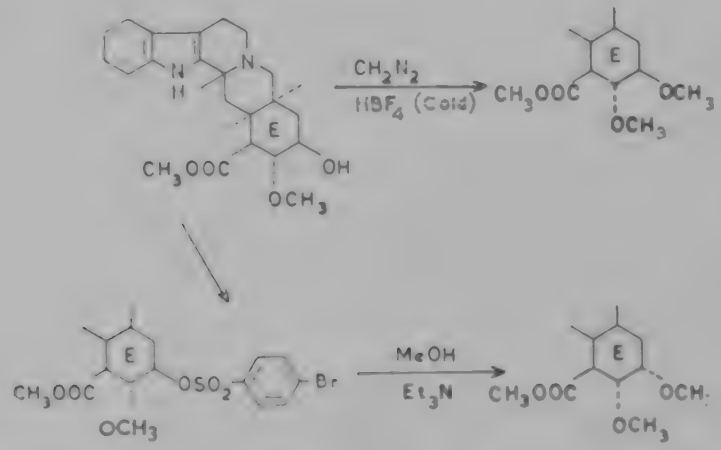
R = CH_3O; = R_2 = TMB; R_3 = H – Raugustine[28]

R = H; = R_2 = TMB; R_3 = H – Isoraunescine[27]

variations in position 18 were the easiest to realize this was the obvious position to modify. A great number of esters have been made, whose activities in the best case equalled, but never surpassed those of reserpine and deserpidine[28]. More refined were experiments to find compounds in which preponderance was given to either sedative or hypotensive activity. Such compounds were found but these specific activities were always reduced and needed higher doses than the parent compounds. Syrosingopine[29] and Su-5171[30] are examples for such compounds but, in general, it must be said that the search for such compounds was not rewarding.



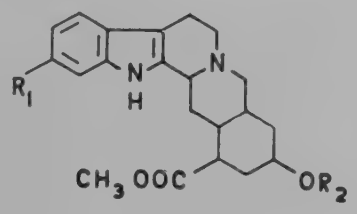
The activity of 18-acetyl methylreserpate (1/3 of reserpine) first demonstrated that there need not be a bulky ester grouping in position 18 and this observation then led to the methylreserpate 18-ethers[31]. Such compounds can be made from methylreserpate either by interaction with CH_2N_2 + HBF or alcoholysis of methylreserpate *p*-bromobenzenesulphonate under mildly alkaline conditions at 100°. In the first instance, a β (normal)-18-ether and in the second case an α (epi)-18-ether results. Both ethers possess definite sedative activity at doses which show practically no hypotensive response.



The above *p*-bromobenzenesulphonate was also treated with dimethyl-sulphoxide in the presence of NMe_3 and thus the 18-ketoreserpate[32] was obtained. Reduction with $NaBH_4$ gave a mixture of β-OH and α-OH methylreserpate,

which could be separated. Pharmacological investigation of the ketone and of methyl 18-epi-reserpate showed a distinct, although reduced sedative activity when compared with reserpine. This finding definitely proved that neither acylation nor alkylation is essential and that in special cases also a keto group can produce sedative activity.

In all the natural analogs of reserpine there is a methoxy or a hydroxy group in position 17 and ethoxy and propoxy analogs have been prepared by total synthesis[33]. The French group has also synthesized 17α-cyano-17-desmethoxy and 17α-methylacetamido-17-desmethoxy reserpine[34], but no pharmacological evaluation of these compounds can be found in the literature. However, a 17-substituent is not essential for activity provided ring E has the appropriate substitution in positions 16 and 18. A 17-desmethoxydeserpidine was already synthesized by Weisenborn[35] in 1957 and was claimed to possess a reduced sedative and hypotensive activity. A similar or perhaps identical compound was obtained by the Czech group in 1965 by means of Woodward synthesis[36] and was found to be hypotensive only.



Weisenborn (1957)

Protiva (1965)

The carbomethoxy group in position 16 is regarded as essential mainly because the compounds lacking this group are inactive. A great number of 16-esters have been prepared[27] and it is, for example, claimed that the dimethylaminoethanol ester possesses sedative but no hypotensive activity[37]. The 16-amide[17] is inactive but the corresponding nitrile (Su-8789)[38] is sedative at 1 mg./kg. orally in the dog.

Stereochemical Considerations

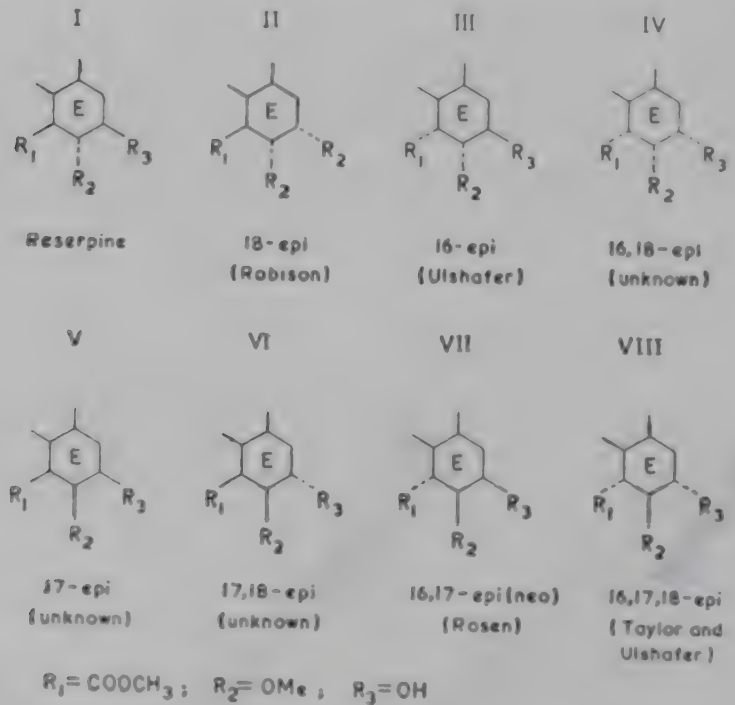
The basic stereochemical puzzles which in their totality have been solved only a few years ago[39] provide some very interesting topics because of their relationship to activity. Only in the discussions of the 18-ethers of methyl reserpate have we so far touched on stereochemical problems and, strangely enough, this is the only case in the structure-activity relations of reserpine where stereochemistry was not crucial. 18β- as well as 18α-alkyl ethers of methyl reserpate show sedative activity. However, as in the steroid field, reserpine activity is connected with a specific shape of the molecule, which serves to remind us that the receptors in the cells must be very selective, i.e. have exacting structural requirements. It is obvious that the shape of the molecule and the stereochemistry of the

substituents in the 16 and 18 positions are of prime importance for the presence or absence of activity.

Carbon atom 3 is the only asymmetric carbon atom not situated in Ring E. Its hydrogen atom is β in reserpine, but by a number of different reactions reserpine can be isomerized to the more stable 3-isoreserpine with a hydrogen in the α position. The influence of this isomerization upon the shape of the alkaloidal molecule is far-reaching; isoreserpine is flat-shaped whereas reserpine has a 'reclining chair shape' which is of paramount importance for activity. Keeping in mind the very specificity of cell-receptors, it was clear that all derivatives of 3-isoreserpine had to be inactive.

Rings D and E of all reserpine alkaloids are *cis*-connected, both hydrogens in 15 and 20 positions being in α. Nothing is known concerning the consequences of changes at this ring junction. Also, no synthetic experiments aimed at obtaining a 15,20 *trans* arrangement have so far been undertaken. This is surprising because an intelligent modification of the Woodward synthesis should yield these new stereoisomers.

At this point let us recapitulate what I have just said about the three asymmetric carbon atoms 3, 15 and 20. All the three have a clearly defined stereochemistry. The hydrogen attached to carbon 3 must be in β-position in order to have an active compound and so far no alkaloids are known or have been synthesized with different stereochemistry in position 15 and 20 whose two hydrogen atoms are both α. The remaining three asymmetric carbon atoms are in positions 16, 17 and 18 and, if 3, 15 and 20 are regarded as being 'fixed', there are eight ring epimers possible, viz.



Of these eight stereoisomeric methyl reserpates, five are known. (II), 18-epi-methylreserpate, possesses sedative activity *per se*, but is inactive when esterified with trimethoxybenzoyl chloride in 18-position. Of all completely esterified stereoisomers, besides reserpine itself, only the di-ester of (VIII) (16, 17, 18 tri-epireserpine) is moderately hypertensive but not sedative. At first sight, any activity would seem to be surprising, but an inspection of the models indicates a possible reason. These models are somewhat difficult to understand. I would therefore like to explain what it is all about. We best look at the two structures at the right of Fig. 2 and imagine that we look at both the structures from a point vertically above ring E. You will notice that in reserpine carbon atoms 16 and 17 and carbon atoms 19 and 20 lie above each other. With 16, 17, 18 tri-epireserpine this is different, because here it is 15/16 and 18/19 which are above each other. In both instances, however, the hexagon of ring E is reduced to a parallelogram which can be drawn as shown on the left of Fig. 2. In each case, the 16 and 18 substituents fall into a certain 'boxed-in' area, that is, the 16, 18 substituents in 16, 17, 18-tri-epireserpine are spatially close to their equivalents in reserpine. This we regard as a necessity for activity as long as no active reserpine analog with different arrangement in ring E has been found.

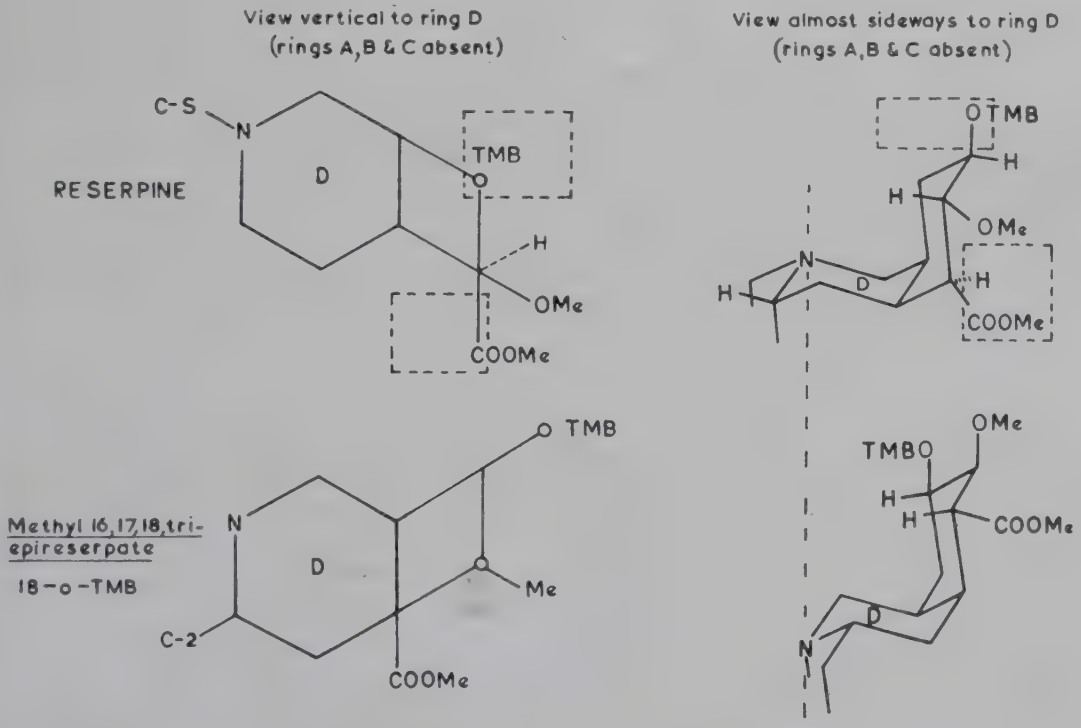


FIG. 2—RELATIONSHIPS BETWEEN RESERPINE AND ITS 16, 17, 18 TRIEPIMER (Hatched areas in reserpine are areas into which the 16-carbomethoxyl and 18-0-substituents must fall for the molecule to show activity)

This may not sound convincing because it is a confusion *per verbaverum*. However, it is an interesting speculation and what I am actually suggesting as the two requirements for activity are: (1) the molecule must be able to assume a kind of a 'reclining chair shape' (3*S*-15*α*-20*α*) and (2) the appropriate C-16 and C-18 substituents must fall into the areas designated by the 'boxed-in' areas shown in Fig. 2.

This concept can accommodate the fact that the naturally-occurring Δ^{18}-reserpine and deserpidine analogs[19] (ranjemidine and deserpidine) are active, as well as our recent findings that the Δ^{18}-17-desmethoxy derivative of reserpine shows sedative and anti-hypertensive properties. The postulated two requirements for activity made us look at models of stereoisomers other than the ones which we have so far discussed. All 3-isoreserpines seem to be excluded, because this molecule is not able to accept a 'reclining chair shape.' The inactivity of the few iso-reserpines (only two are known) confirms the correctness of this statement. If we go one step further and build models of reserpines with *trans* D/E ring, derivatives of ψ-yohimbine should be considered. In these compounds the 16-$COOCH_3$ group falls within the boxed area if this group is in α-position but of the two possible orientations for the 18-ester group the most favourable one, the β, does not meet the requirements. Therefore, no activity could be expected. However, these are mere speculations and we would be only too happy if the isolation of such a new stereoisomer would confirm our theories. Evidently, much work still remains to be done but we think that we are getting closer to an exact definition of the molecular parameters which make this classic drug so important.

I am grateful to Dr W. I. Taylor of CIBA Pharmaceutical Company for the many discussions and help in writing this paper.

REFERENCES

1. Mueller, J. M., Schlittler, E. & Bein, H. J., *Experientia*, **8** (1952), 338.
2. For reviews see R. A. Lucas in "Progress in Medicinal Chemistry" (G. P. Ellis and G. B. West, eds.) p. 146. Butterworths, Washington and London 1963; E. Schlittler and A. J. Plummer in "Psychopharmacological Agents" (Maxwell Gordon, ed.) Vol. I. p. 9. Academic Press, New York, 1964; E. Schlittler in "Rauwolfia: Botany, Pharmacognosy, Chemistry and Pharmacology". Little Brown and Co., Boston, Toronto 1957; C. Viel, *Ann. Pharm. Fr.*, **20** (1962), 907.
3. Schlittler, E. in "Alkaloids" (R.H.F. Manske, ed.) Vol. 8., p. 287, Academic Press, New York 1965; Taylor, W. I. "Indole Alkaloids" (Sir Robert Robinson, ed.) Pergamon Press, London and New York, 1966.
4. Woodward, R. B., Bader, F. E., Bickel, H., Frey A. J. & Kierstad, R., *J. Am. Chem. Soc.*, **78** (1956), 2023; *Tetrahedron*, No. 2 (1958), 1.
5. Velluz, L., Muller, G., Joly, R., Nominé, G., Allais, A., Warnant, J., Bucourt, R. & Jolly, J., *Bull. Soc. chim. Fr.*, (1958), 145.
6. Petrzilka, T., Frey, A., Hofmann, A., Ott, H., Schenk, H. & Troxler, F. (Sandoz Ltd) US Pat. 2,959,591 (1960); Belgian Pat. 556,281 (1958).

7. The Czech publications are so numerous that no special reference can be quoted. Most of these publications can be found in *Colln Czech. chem. Commun.*, starting in 1957.
8. Brodie, B. B. in "5-Hydroxytryptamine" (G. P. Lewis, ed.) p. 64, Pergamon Press, Lond. 1958; Brodie, B. B. & Shore, P. A., *Ann. N. Y. Acad. Sci.*, **66** (1957), 631.
9. Pletscher, A., Burkard, W. P., Bruderer, H. & Gey, K. F., *Life Sci.*, **2** (1963), 828.
10. Gal, E. M., Drewes, P. A. & Barraclough, C. A., *Abstr. First int. Pharmac. Meet., Stockholm*, 1961, p. 32, Pergamon Press, London, 1961.
11. Weisenborn, F. L., US Pat. 2,912,436 (1959).
12. Ulshafer, P. R., Nugent, R. H. & Dorfman, L., *Chem. Ind.* (Lond.), (1962), 1863.
13. Peterfalvi, M. & Jequier, R., *Arch. int. Pharmacodyn.*, **124** (1960), 237.
14. Velluz, L., Peterfalvi, M. & Jequier, R., *C. r. Acad. Sci.*, **247** (1958), 1905.
15. Emden, A. v. d., Knick, B. & Wagner, H. J., *Arzneimittel-Forsch.*, **14** (1964), 905; see also *Br. med. J.*, (1962), 875.
16. Velluz, L., Muller, G., Joly, R., Nomine, G., Mathieu, J., Allais, A., Warnant, J., Valls, J., Bucourt, R. & Jolly, J., *Bull. Soc. chim. Fr.*, (1958), 673; Muller, G. & Allais, A., *Naturwiss.*, **47** (1960), 82; Petrzilka, T., Frey, A., Hofmann, A., Ott, H., Schenk, H. R. & Troxler, F. (Sandoz Ltd) Union of South Africa Patent Application 1,247, 158 (1958); Protiva, M., Rajsner, M. & Jilek, J. O., *Monatsh. d. Chemie*, **91** (1960) 703; Novak, L. & Protiva, M., *Naturwiss.*, **46** (1959), 579.
17. Huebner, C. F., *J. Am. chem. Soc.*, **76** (1954), 5792; see also Berthaux, P. & Newman, M., *Arzneimittel-Forsch.*, **14** (1964), 1040.
18. Jirkovsky, I. & Protiva, M., *Colln Czech. chem. Commun.*, **28** (1963), 2582.
19. Velluz, L., Muller. G. & Allais, A., *C. r. Acad. Sci.*, **247** (1958), 1746; see also 25.
20. Dorfman, L., Furlenmeier, A., Huebner, C. F., Lucas, R. A., MacPhillamy, H. B., Mueller, J. M., Schlittler, E., Schwyzer, R. & St. Andre, A. F., *Helv. chim. Acta*, **37** (1954), 59.
21. Weichet, J., Kakac, B. & Blaha, L., *Colln Czech. chem. Commun.*, **27** (1962), 843; Blaha, L., Kakac, B. & Weichet, J., *ibid.*, **27** (1962), 857.
22. Schlittler, E. in "Alkaloids" (R.H.F. Manske, ed.), Vol. 8, p. 291, Academic Press, New York (1965).
23. Klohs, M. W., Keller, F., Williams, R. E. & Kusserow, G. W., *J. Am. chem. Soc.*, **79** (1957), 3763.
24. Popelak, A., Haack, E., Lettenbauer, G. & Spingler, H., *Naturwiss.*, **48** (1961) 73; Popelak A. & Lettenbauer, G., *Arch. Pharm.*, **296** (1963), 261.
25. Hosansky, N. & Smith, E., *J. Am. Pharm. Ann. Sci.*, **44** (1955), 639; Huebner, C. F. & Schlittler, E., *J. Am. chem. Soc.*, **79** (1957), 250.
26. Mueller, J. M., *Experientia*, **13** (1957), 479.
27. Lucas, R. A., Kiesel, R. J. & Ceglowski, M. J., *J. Am. chem. Soc.*, **82** (1960), 493.
28. Lucas, R. A., Kuehne, M. E., Ceglowski, M. J., Dziemian, R. L. & MacPhillamy, H. B., *J. Am. chem. Soc.*, **81** (1959), 1928.
29. Plummer, A., Barrett, W. E., Maxwell, R. A., Finocchio, D., Lucas, R. A. & Rutledge, R., *Abstr. Meet. Am. Soc. Pharmac. exp. Ther.*, Ann Arbor (Mich.) 1958.
30. Garattini, S., Mortari, A., Valsecchi, A. & Valzelli, L., *Nature*, **183** (1959), 1273.
31. Robison, M. M., Lucas, R. A., MacPhillamy, H. B., Barrett, W. & Plummer, A. J., *Experientia*, **17** (1961), 14; Robison, M. M., Lucas, R. A., MacPhillamy, H. B., Dziemian, R.L., Hsu, I. & Kiesel, R. J., *J. Am. chem. Soc.*, **83** (1961), 2694; Popelak, A. & Lettenbauer, G., *Arch. Pharm.*, **295** (1962), 427.
32. Robison, M. M., Pierson, W. G., Lucas, R. A., Hsu, I. & Dziemian, R. L., *J. org. Chem.*, **28** (1963), 768.
33. Petrzilka, T., Frey, A., Hofmann, A., Ott, H., Schenk, H. R. & Troxler, F., (Sandoz Ltd) Union of South Africa Patent Application 1,247,158, (1958).
34. Muller, G. & Allais, A., *Naturwiss.*, **47** (1960), 82.

F. L., *ibid.*, **79** (1957), 4818.
36. Lahiri, L., Julek, J. O., Jirkovsky, J. & Protiva, M., *Collect. Czech. chem. Commun.*, **30** (1965), 2236.
37. Lucas, R. A., Krikel, R. J. & Cieslowski, M. J., *J. Am. chem. Soc.*, **82** (1960), 193.
38. Plummer, A., private communication (May 13, 1960).
39. Aldrich, P. E. *et al.*, *J. Am. chem. Soc.*, **81** (1959), 2481.
40. Ulchester, P. R., Pandow, M. L. & Nugent, R. H., *J. org. chem.*, **21** (1956), 923; Shamma, M. & Walker, E. F., *Chem. Ind. (Lond.)*, (1962), 1896; Shamma, M. & Slusarchyk, R. J., *Tetrahedron Letters*, No. 33, (1964), 2277; Smith, E., Jaret, R. S., Shamma, M. & Slusarchyk, R. J., *J. Am. chem. Soc.*, **86** (1964), 2083.

Amines of the 10,11-Dihydrodibenzo (*b,f*) thiepine Series : A New Group of Multipotent Neurotropic and Psychotropic Substances

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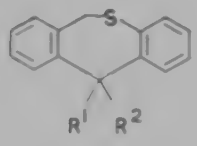
One of the most important lines of searching for new psychotropic drugs starts with the conception that these substances are the basic derivatives of tricyclic systems in which the side chain, carrying the basic function, is attached to the central ring of the system. It is an empirical conception based on accidental discoveries of very active substances, e.g. chlorpromazine and imipramine as basic representatives of neuroleptics and thymoleptics. A systematic variation of the structure of these basic compounds led to the synthesis and pharmacological testing of thousands of substances and, at the same time, to the finding of certain rules in the relationship between structure and activity enabling at least a partially rational approach in the preparation of further substances.

Our group at the Pharmaceutical and Biochemical Research Institute in Prague studied intensively during the last 6 years the basic derivatives of 6,11-dihydrodibenzo (*b, e*) thiepine (1), i.e. of a tricyclic linearly condensed system with two external aromatic nuclei and a central seven-membered dihydrothiepine cycle carrying, in the position 11, the amino group or a side chain terminated with the amino group. This study yielded several interesting substances, some of which will very probably find practical use in the therapy. In the first line there are prothiadene (2), northiadene (3) and hydrothiadene (4), having antireserpine activity. The first of them which is a simple thia analog of amitriptyline, has been under clinical

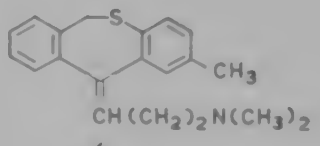
myeitigation since 1961. It may be classed as a good antidepressant for light and medium non-psychotic depressions of various etiology; because it produced no side effects, it is also suitable for out-patients. Clinical testing of the further two drugs has recently been started. The work led to the finding of several highly active antihistaminics, especially amethoxepine (5), a basic ether, and finally methiadene (6), the 2-methyl derivative of prothiadene, giving very promising clinical results and suitable for parenteral use.

In the last two years, the centre of our interest shifted to the group of derivatives of the isomeric 10,11-dihydrodibenzo [*b*, *f*] thiepine (7). Till now the literature mentioned only two attempts to find neurotropic substances between derivatives of the corresponding 10, 11-unsaturated system, dibenzo [*b*, *f*] thiepine (8). Both attempts are described only in patent literature and it is difficult to estimate the real therapeutic value of the compounds described. First is a patent of Smith, Kline and French Laboratories (1963) describing 10-dialkylaminoalkyl dibenzo [*b*, *f*] thiepines (9) for which central stimulant, antihistaminic and antispasmodic activities are given. The second is a recent patent application of Geigy disclosing the synthesis of 10-(*a*-aminoalkyl) dibenzo [*b*, *f*] thiepines (10) having adrenolytic and central depressant activity.

Our work in the series of the 10,11-saturated compounds, carried out mainly by Jilek, started with the synthesis of 10-oxo-10,11-dihydrodibenzo [*b*, *f*] thiepine (16). Diphenylsulphide-2-carboxylic acid (11), easily accessible by the reaction of *o*-iodobenzoic acid with thiophenol, was reduced with lithium aluminum hydride to *o*-hydroxymethyldiphenylsulphide (12). By treatment with thionyl chloride in pyridine, *o*-chloromethyl-



1, $R^1 = R^2 = H$
2, $R^1R^2 = CH(CH_2)_2N(CH_3)_2$
3, $R^1R^2 = CH(CH_2)_2NHCH_3$
4, $R^1 = H$, $R^2 = (CH_2)_3N(CH_3)_2$
5, $R^1 = H$, $R^2 = O(CH_2)_2N(CH_3)_2$



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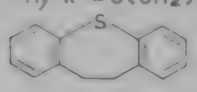
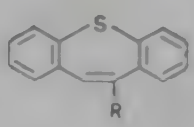
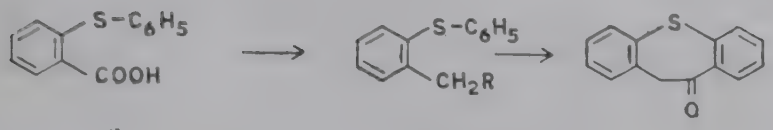


7



8, R = H
9, $R = (CH_2)_nNR^1R^2$
10, $R = CHR^1-NR^2R^3$



11

12, R = OH
13, R = Cl
14, R = CN
15, R = COOH

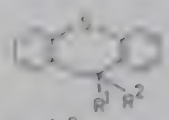
16

diphenylsulphide (13) was obtained giving with sodium cyanide in aqueous methanol, the nitrile (14). Alkaline hydrolysis afforded diphenylsulphide-2-acetic acid (15), the cyclization of which gave smoothly the required ketone (16) (m.p. 72–73°, 1680 cm.$^{-1}$).

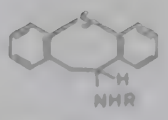
In the first line we tried to prepare a (*b*, *f*) analog of the mentioned prothiadene. Reaction of the ketone (16) with 3-dimethylaminopropylmagnesium chloride took, however, a very unfavourable course, evidently caused by a high degree of enolization of the ketone. In a yield of only 3 per cent the required amino alcohol (17) was obtained and dehydrated then with acetyl chloride in boiling chloroform. For the product the structure of an aromatic dibenzo (*b*, *f*) thiepine derivative was expected but the spectra excluded this formulation and favoured the isomeric structure (18) with an exocyclic double bond. The substance is thus a true position isomer of prothiadene from which it differs greatly in the pharmacodynamic properties; it has neither antireserpine nor central depressant activity.

In our further work we studied amines with the amino group attached directly to the position 10 of the skeleton. For their preparation, three different approaches were used. Reduction of the oxime (19) proved unsuitable: attempts to reduce with sodium and ethanol or with lithium aluminum hydride were unsuccessful, and only reduction with zinc and boiling acetic acid gave in low yield directly the corresponding 10-acetamido derivative (20). More successful was the Leuckart reaction of the ketone (16) giving satisfactory yield of the 10-formamido derivative (21). This compound was further transformed, on the one hand, by hydrolysis to the primary amine, 10-amino-10, 11-dihydrodibenzo (*b*, *f*) thiepine (22), on the other by reduction with lithium aluminum hydride to the secondary amine, 10-methylamino-10, 11-dihydrodibenzo (*b*, *f*) thiepine (23). The hydrochlorides of these simple amines display a weak but distinct central depressant activity.

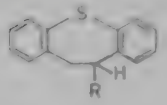
The third method of synthesis of amines of this type started with reduction of the ketone (16) with sodium borohydride to 10-hydroxy-10, 11-dihydrodibenzo (*b*, *f*) thiepine (24) (m.p. 100°). This substance, treated with thionyl chloride or hydrogen chloride, gave 10-chloro-10, 11-dihydrodibenzo (*b*, *f*) thiepine (25) which proved to be a very useful intermediate. Under partial dehydrochlorination to dibenzo (*b*, *f*) thiepine (8) it reacts with amines giving the corresponding 10-amino derivatives.



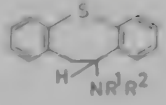
16, $R^1R^2 = O$
17, $R^1 = H, R^2 = (CH_2)_3N(CH_3)_2$
18, $R^1R^2 = CH(CH_2)_2N(CH_3)_2$
19, $R^1R^2 = NOH$



20, $R = COCH_3$
21, $R = CHO$
22, $R = H$
23, $R = CH_3$

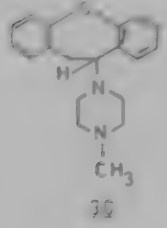


24, $R = OH$
25, $R = Cl$



26, $R^1 = R^2 = CH_3$

27, R^1R^2 =



30

28, R^1R^2 =

29 $R^1 = H, R^2 = (CH_2)_2N(C_2H_5)_2$

In this way condensations with dimethylamine, piperidine, norpethidine, and 2-diethylaminoethylamine were carried out leading to the corresponding amines (26–29) showing also some indications of central depressant activity.

A completely unexpected turn in our work was brought about by the product of condensation of the chloro derivative (25) with *N*-methylpiperazine. It is 10-(4-methylpiperazino)-10, 11-dihydrodibenzo (*b*, *f*) thiepine (30) (m.p. 135°, maleate m.p. 158°) called perathiepine. In experiments carried out by Metysova in our Pharmacological Department in mice, rats and rabbits this compound revealed a surprisingly strong central depressant action. Motor coordination disturbances in the rotating rod test in mice developed within 5 min. after its intravenous administration, their maximum was reached 15 min. after the injection and these changes lasted for more than 1 hr. The mean effective dose of perathiepine was 187 mcg./kg. The spontaneous motor activity of mice, investigated by the photocell method by Dews, was also reduced by perathiepine. The dose which decreased the animals' activity by 50 per cent of the control values was 156 mcg./kg. Likewise a significant prolongation of thiopental sleeping time in mice and rats and an intensive hypothermic action in mice were found after the administration of perathiepine. In the majority of tests mentioned, perathiepine was approximately 2–3 times as active as chlorpromazine. The phenmetrazine toxicity in aggregated mice was also reduced by perathiepine.

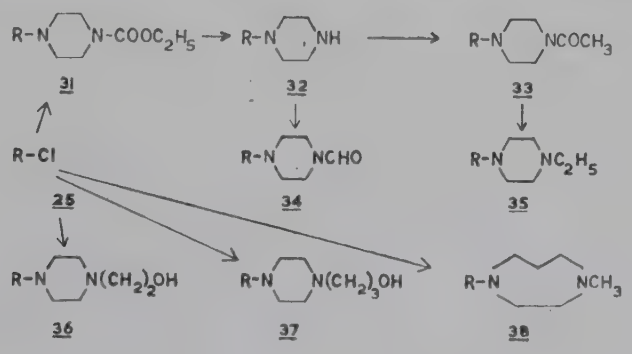
Perathiepine showed further a protective action in the supramaximal electroshock procedure in mice in a relatively high dosage. It increased the occurrence of convulsions and the toxicity of pentetrazole in mice. Some reserpine effects in mice (eye-lid ptosis) and rats (ulcerogenic effect) were increased after the administration of perathiepine. The antihistaminic activities of perathiepine and cyproheptadine in guinea-pigs *in vivo* hista-

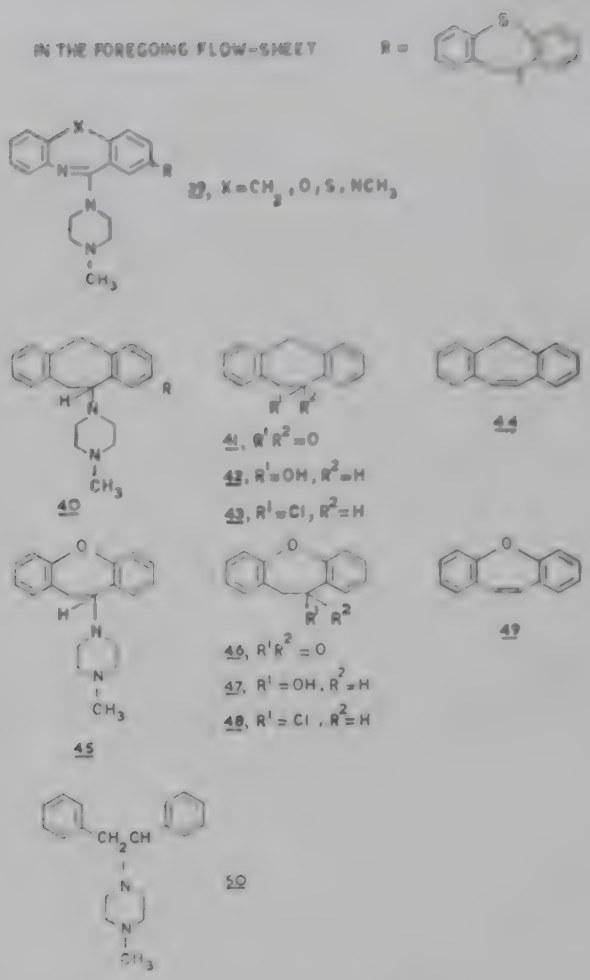
mine aerosol, histamine detoxication) were equal. The antiserotonine action of perathiepine in rats *in vivo* was higher than that of cyproheptadine, but weaker *in vitro*. The medium intravenous lethal dose of perathiepine in mice was 42·3 mg./kg.; lower doses caused paralysis of the extremities and powerful sedation and higher doses clonic convulsions, dyspnoea and exitus. In general perathiepine may be classed as an effective neuroleptic drug, exceeding in the majority of tests the activity of chlorpromazine and with pronounced antihistamine and antiserotonine actions.

The exceptionally high pharmacodynamic activity of perathiepine (30) led us to extend the synthetic work to the area of closest analogs. At first were studied the analogs derived by variation in the piperazine part of the molecule, only by the substitution of the methyl group. Condensation of the chloride (25) with *N*-(ethoxycarbonyl) piperazine gave the carbamate (31), affording by hydrolysis with potassium hydroxide in ethylene glycol at 180–90° 'norperathiepine' (32), the next lower homolog of perathiepine. This compound may be used as starting material for the synthesis of higher perathiepine homologs: acetylation gave the acetyl derivative (33) which yielded by reduction with lithium aluminum hydride 10-(4-ethylpiperazino)-10, 11-dihydrodibenzo (*b*, *f*) thiepine (35), the next higher homolog of perathiepine. Heating of norperathiepine (32) with ethyl formate in autoclave to 120–30° gave the formyl derivative (34), affording on lithium aluminum hydride reduction perathiepine (30) which thus represents a second synthesis of this substance.

Condensations of the chloride (25) with *N*-(2-hydroxyethyl) piperazine and *N*-(3-hydroxypropyl) piperazine gave substances (36) and (37) which were expected to be very active based on experiences with the phenothiazine series. By treatment of the chloride (25) with 1-methylhexahydro-1, 4-diazepine, 'homoperathiepine' (38) was obtained representing, however, a further type of perathiepine analogs in which the whole piperazine residue is substituted by another amine. Some simple analogs of this type have already been mentioned earlier in this paper.

The structure of perathiepine is evidently related to the structure of a new series of neuroleptics represented by the general formula (39) and described very recently by the group of Wander. This fact led us to prepare three perathiepine analogs with modified skeletons. The first was 10-(4-





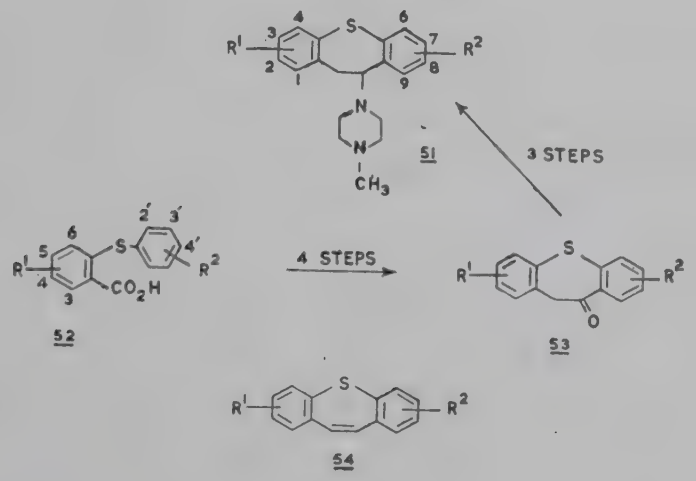
methylpiperazino)-10,11-dihydrodibenzo(*a,d*)cycloheptene (40), derived from perathiepine by substitution of the sulphur atom in the ring by a methylene group. The synthesis was carried out from the known 10-oxo-10, 11-dihydrodibenzo(*a, d*)cycloheptene (41) which was reduced with sodium borohydride to the alcohol (42). Treatment with thionyl chloride affords the corresponding chloride (43), which is, however, so unstable that it could not be prepared in analytically pure state. Already when crystallized from petroleum ether it loosened hydrogen chloride and gave the unsaturated dibenzo(*a, d*)cycloheptene (44). It may, however, be condensed in crude state with *N*-methylpiperazine, and in this way the required perathiepine analog (40) was obtained.

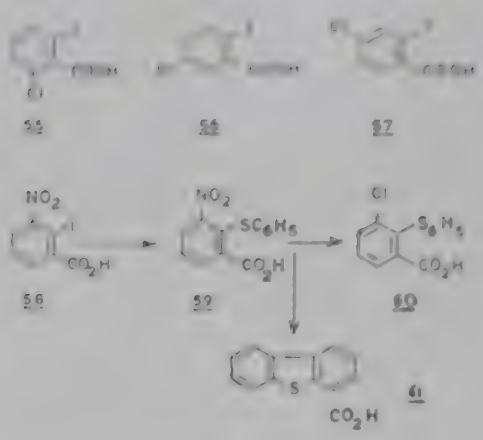
We synthesized further 10-(4-methylpiperazino)-10, 11-dihydrodibenzo(*b, f*)oxepine (45), derived from perathiepine by substitution of the sulphur by oxygen. Its synthesis was carried out in a similar way again from the known ketone (46) which was first reduced to the alcohol (47). Treatment with hydrogen chloride gave the chloride (48), affording, by treatment with *N*-methylpiperazine, the required amine (45), along with the unsaturated dibenzo(*b, f*)oxepine (49). Finally, as the last analog of this type 1-(4-methylpiperazino)-1,2-diphenylethane (50) was prepared by

condensation of the known 1,2-diphenylethyl chloride with *N*-methylpiperazine. This substance is an open *des*-thia analog of perathiepine. This part of work was carried out experimentally by Seidlova and Jirkovsky.

Our further work was aimed at a study of systematic substitution of the molecule of perathiepine in various positions of the tricyclic skeleton, primarily in the benzene nuclei, as shown in the general formula (51). This work is still in progress and I shall, therefore, mention only the synthesis and activity of halogenated derivatives of perathiepine (51, R^1 and/or R^2=halogen atoms). Greatest attention was paid to the Ar-monochloro perathiepines: my coworkers Jilek, Ernest, Adlerova and Pelz prepared, meanwhile, seven of the eight theoretically possible isomers, the 1-, 2-, 3-, 4-, 6-, 7-, and 8-chloro derivatives. These compounds were synthesized similarly to perathiepine itself: we started with the correspondingly chlorinated diphenylsulphide-2-carboxylic acids (52) which by homologization (via alcohols, chlorides and nitriles) and by the following cyclization, i.e. in four steps, were transformed to the chlorinated ketones (53). Subsequent three steps (reduction to alcohols, treatment with hydrogen chloride, and condensation with *N*-methylpiperazine) afforded the required chlorinated perathiepines. By-products were the corresponding chlorinated dibenzo (*b*, *f*) thiepines (54), formed by concomitant dehydrochlorination.

The synthesis of the starting chlorinated diphenylsulphide-2-carboxylic acids (52) was mostly not a serious problem. In most cases, it consisted in condensations of *o*-iodobenzoic acid or its chloro derivatives with thiophenol or its chloro derivatives. This was the case of the 3-, 4-, 5-, 2′-, 3′-, and 4′-chloro derivatives. During this work, syntheses of the new 6-chloro-2-iodo- (55), 5-chloro-2-iodo- (56) and 4-chloro-2-iodo-benzoic acid (57) were elaborated. In the 4-chloro-perathiepine synthesis, the known 2-iodo-3-nitrobenzoic acid (58) was condensed with thiophenol. The prepared 3-nitro-2-phenylthiobenzoic acid (59) was then transformed to the required 6-chlorodiphenylsulphide-2-carboxylic acid (60) by reduction with





ferrous hydroxide and subsequent Sandmeyer reaction. This last step was complicated by a cyclization of the type of Gomberg reaction giving as a by-product dibenzothiophene-4-carboxylic acid (61) in large quantity.

Finally, the cyclization of 3′-chlorodiphenylsulphide-2-acetic acid (62) is perhaps worth mentioning. As products, the isomeric ketones (63) and (64) could be expected or a mixture of both. The infrared spectrum of the product showing strong absorption bonds at 818, 831 and 871 cm.$^{-1}$ (E. Svátek) proved that the 7-chloro ketone (63) was formed almost exclusively.

The syntheses of the 8-fluoro- (65), 8-bromo- (66), and 2, 8-dichloro- (67) derivatives of perathiepine were carried out similarly as the syntheses of the 2- and 8-chloro perathiepines.

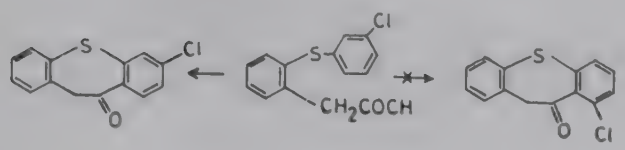
As potentially interesting compounds, the 2-dimethylaminoethyl ethers (68) were prepared from the corresponding alcohols (intermediates of the synthesis of perathiepine and derivatives) by treatment with sodium amide and 2-dimethylaminoethyl chloride.

Our systematic chemical and pharmacological investigations in the field of perathiepine analogs are continuing. At this moment I am able to give only a preliminary summary of the relationship between structure and central depressant activity in this group.

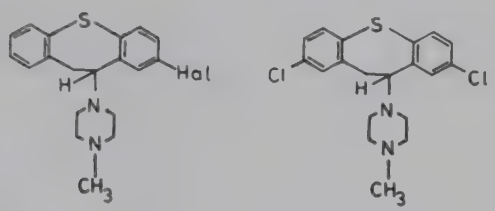
(1) The substitution of the methylpiperazine part of the molecule by residues of simpler amines, e.g. of methylamine, dimethylamine, piperidine, and 2-diethylaminoethylamine decreases the central depressant activity in a decisive manner.

(2) The removal of the *N*-methyl group leading to norperathiepine is connected with reduction of activity to one half of that of perathiepine. In the rotating-rod test, norperathiepine is still more active than chlorpromazine.

(3) The substitution of the *N*-methyl group with 2-hydroxyethyl or 3-hydroxypropyl is connected likewise with a slight drop of activity. Nevertheless the 3-hydroxypropyl compound is twice as active as chlorpromazine.

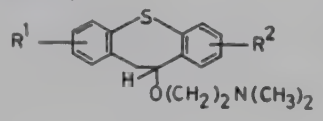


63 62 64



65, Hal = F
66, Hal = Br

67



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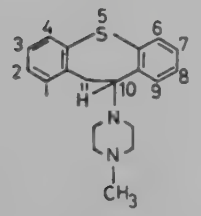

PERATHIEPINE

(4) The substitution of the *N*-methylpiperazine part with a methylhomopiperazine residue brings about a surprisingly strong decrease of activity (to approximately 8 per cent perathiepine activity).

(5) The substitution of the *N*-methyl with ethoxycarbonyl or acyl is connected with an almost complete loss of activity.

(6) From the substitutions studied till now, only substitution in position 8 appears to be favourable. The 8-chloro derivative of perathiepine ('octoclothepine') is approximately 3 times as active as perathiepine, i.e. ten times as active as chlorpromazine and surpasses the activity of chlorprothixene. Chlorination in other positions decreases the potency in strongly varying degrees; at least in position 2 (70 per cent of perathiepine activity), at most in positions 3, 4 and 6 (2–5 per cent of perathiepine activity).

(7) The modifications of the skeleton, studied till now, had generally no favourable influence. The presence of the sulphur atom in position 5 seems to be connected with the highest activity. The methylene analog of perathiepine retains 70 per cent of the perathiepine activity and the oxygen analog only 30 per cent. The open *des*-thia analog is practically inactive.

(8) A rather high degree of central depressant activity is also shown by 2-dimethylaminoethyl esters derived from the corresponding 10-hydroxy-10, 11-dihydrodibenzo (*b*, *f*) thiepines. The unsubstituted compound has approximately 10 per cent of the perathiepine activity; the most active compound of the series, the 8-chloro derivative ("amethoclothepine") has 70 per cent and exceeds thus the activity of chlorpromazine.

At the end I would like to point out that the discovery of the high neuroleptic activity in the group of dibenzo (*b*, *f*) thiepine derivatives was a surprise. The existing knowledge indicated that the highest neuroleptic activity required (a) a tricyclic system with a six-membered middle ring, and (b) a basic amino group separated from the skeleton by a three-carbon chain attached to the central ring. The structures of perathiepine and analogs differ from these requirements and represent thus really a new type. We have now to await the results of the clinical testing of perathiepine which has just started. The true therapeutic value of this series remains, of course, uncertain in the meantime.

The Role of Trimethoxybenzene Ring in Psychotropic Drugs

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The knowledge regarding the possession of activity of trimethoxybenzene ring came with the isolation and determination of the chemical structure of mescaline. Subsequently the discovery of reserpine attracted considerable attention from pharmacologists and medicinal chemists. In the beginning, the fact that the main structure of reserpine was based upon the indole skeleton, led many workers to the synthesis of simpler indole derivatives as reserpine analogues (Benington *et al.*, 1958; Karim *et al.*, 1960 a & b). But at reasonable dosage levels, none of these compounds was comparable in their activity to reserpine. It is worthwhile to mention here that, perhaps with the solitary example of reserpine itself, invariably all the pharmacologically active plant principles having indole structure are psychotomimetic or central stimulants. Important examples are *N*, *N*-dimethyltryptamine (Szara, 1956; Sai-Halaszy *et al.*, 1958), bufotenine (Fabing and Hawkins, 1956), psilocybin (Hofmann *et al.*, 1959), α-methyltryptamine (Murphee *et al.*, 1960), harmine (Pennes and Hoch, 1957) and ibogaine (Schneider and Sigg, 1957) all of which possess hallucinogenic property. Other indole derivatives like nitroindole, aminoindole and medmain tested by Woolley and collaborators (Woolley and Shaw, 1957) also proved to be hallucinogens.

Some of the drugs possessing the trimethoxybenzene (TMB) structure are shown in Fig. 1.

Of these drugs, reserpine, colchicine and mescaline are naturally occurring drugs while trimeglamide, tigan and 3, 4, 5-trimethoxy amphetamine are synthetic drugs. The last two drugs asarone and β-asarone have only been recently worked out (Dandiya and Sharma, 1962). Our attention was drawn to the trimethoxybenzene structure when we were studying the pharmacology of the volatile oil of *Acorus calamus*, a drug commonly used in India in the Ayurvedic system of medicine for various mental ailments (Dandiya *et al.*, 1959a, Dandiya and Cullumbine, 1959; Dandiya

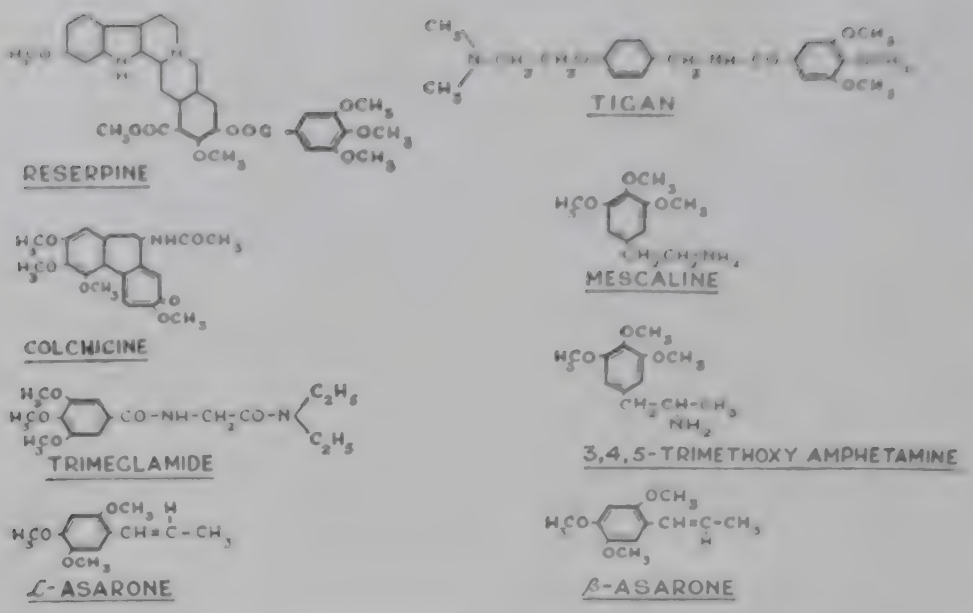


FIG. 1 — WELL-KNOWN DRUGS POSSESSING TRIMETHOXYBENZENE RING

et al., 1959b). The finding that the pharmacological actions of the volatile oil of *Acorus calamus* were due to asarone and β-asarone which are *trans* and *cis* isomers, respectively, of 2, 4, 5-trimethoxy-1-propenyl benzene led us to do more work on similar compounds. In recent years, we have studied the pharmacology of asarone and β-asarone in greater detail (Dandiya and Menon, 1963; Dandiya and Menon, 1964; Dandiya and Menon, 1965) and a few salient features regarding the psychopharmacological properties of asarone are shown in Table 1.

The pharmacological actions of asarone were in many cases similar to those of reserpine and chlorpromazine but the action of asarone differs to some extent from these drugs as regards its protective action against electroconvulsions and from reserpine in its failure to deplete 5-HT level of brain and also because of its failure to cause stimulation in animals treated earlier with iproniazid. We have shown that asarone has a quicker onset of action in monkeys than reserpine and also that its action lasts longer than chlorpromazine in these animals.

Our findings regarding the higher potency of this naturally occurring substance having 2, 4, 5-trimethoxy-1-propenyl benzene has led us to do some rethinking on the role of the trimethoxybenzene nucleus in psychotomimetic and tranquillizing agents. Since a considerable amount of work has been done on the role of the indole nucleus in these structures we have tried to classify some of the simple derivatives of indole and trimethoxy compounds in Table 2.

It is interesting to note here that both indole and TMB derivatives have one property in common, viz. minor variation of their chemical structure leads either to the development of tranquillizing or a hallucinogenic property. Here, each of these two is divided into group A (tran-

TABLE 1—COMPARISON OF PHARMACOLOGICAL ACTIONS OF ASARONE WITH RESERPINE AND CHLORPROMAZINE

S. No.	Effects on	Asarone	Reserpine	Chlorpromazine
1.	Spontaneous activity in rats, mice and monkeys	Reduction	Reduction	Reduction
2.	Barbiturate hypnosis	Potentiation	Potentiation	Potentiation
3.	Behaviour of cats, monkeys	Taming	Taming	Taming
4.	Fighting behaviour of mice	Suppression	Suppression	Suppression
5.	Amphetamine toxicity in aggregated mice	Protection	Protection	Protection
6.	Conditioned avoidance response	Specific blockade	Specific blockade	Specific blockade
7.	Choice discrimination behaviour	Anxiety reduction	Anxiety reduction	Anxiety reduction
8.	Rectal temperature	Hypothermia	Hypothermia	Hypothermia
9.	LSD-25 induced hyperthermia	Antagonism	Antagonism	Antagonism
10.	Electro-convulsions	Protection (?)	Facilitation	Facilitation (?)
11.	Iproniazid-treated mice	Sedation	Stimulation	Sedation
12.	Brain 5-HT level	No change	Reduction	No change
13.	Hyperactivity due to:			
	(a) LSD-25	Antagonism	Antagonism	Antagonism
	(b) Methylphenidate	Antagonism	Antagonism	Antagonism
	(c) *d*-Amphetamine	Antagonism	Antagonism	Antagonism
	(d) Imipramine	Partial antagonism	Antagonism	—
	(e) Mescaline	Antagonism	Reduction (?)	Antagonism
14.	Adrenal ascorbic acid	Depletion (with large doses)	Depletion	Depletion
15.	Stress-induced adrenal ascorbic acid depletion	Prevention	Prevention	Prevention
16.	Stress-induced adrenal adrenaline depletion	Prevention	Prevention	Prevention
17.	Stress-induced brain neurohormonal changes	Prevention	Prevention	Prevention
18.	Tremorine-induced tremors	Partial protection	Partial protection	Partial protection
19.	α-Methyl tyrosine treated animals:			
	(a) Pentobarbitone hypnosis	Further potentiation	Further potentiation	Further potentiation
	(b) Conditioned avoidance response	do	do	do
	(c) Rectal temperature	do	do	do
20.	Blood pressure	Hypotension	Hypotension	Hypotension
21.	Smooth muscle contractions induced by:			
	(a) Acetylcholine	Antagonism	Antagonism	Antagonism
	(b) Histamine	Antagonism	—	Antagonism
	(c) 5-Hydroxytryptamine	Antagonism	Antagonism (?)	Antagonism

TABLE 2—PHARMACOLOGICAL ACTIONS OF SIMPLE DERIVATIVES OF INDOLE AND TRIMETHOXYBENZENE

S.No.	Effects on	Indole derivatives		Trimethoxybenzene derivatives	
		Group A*	Group B†	Group A**	Group B‡
1.	Alertness	—		—	
2.	Spontaneous activity	—		—	
3.	Temperature	—	+	—	
4.	Pupil	Constriction	Dilatation	Constriction	Dilatation
5.	Eyelid	Ptosis	Exophthalmus	Ptosis	Exophthalmus
6.	Gross behaviour	Sedation	Aggressiveness	Sedation	Aggressiveness
7.	Barbiturate hypnosis	Potentiation	Antagonism	Potentiation	Potentiation by mescaline
8.	Erection of hair	No effect	Piloerection	No effect	
9.	Conditioned avoidance response	Blockade	Disruption	Blockade	
10.	Amphetamine excitation			Antagonism	Potentiation
11.	LSD-Excitation		Potentiation	Antagonism	Potentiation
12.	Analgesia				
13.	Threshold to electric shock	Increase		Increase or no effect	Increase by mescaline
14.	EEG		Low voltage fast activity		Low voltage fast activity
15.	Anti-5HT effect	BAS-Present	Present		
16.	Blood pressure	5-HT Biphasic BAS-Hypotensive		Hypotension	Hypotension

*5-HT, BAS

†Nitroindole, aminoindole, Medmain bufotenine

**Asarone, β-asarone and many others

‡Mescaline, trimethoxy-amphetamine, S.E.

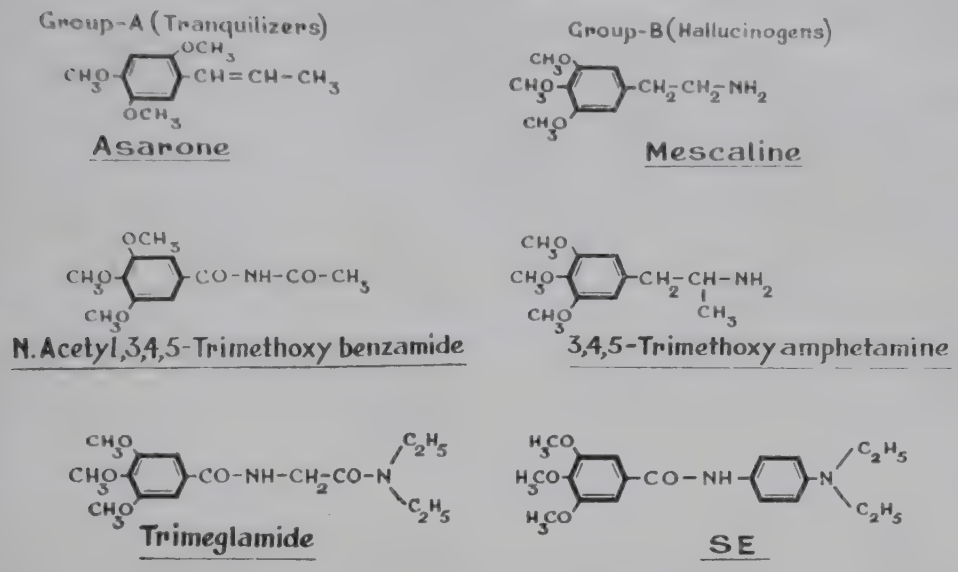


FIG. 2—TRIMETHOXYBENZENE DERIVATIVES

quillizers) and group B (hallucinogens). Group A of indole derivatives is represented by 5-HT and BAS (benzyl analogue of serotonin) whose actions compare well with group A of TMB, namely asarone and β-asarone. Similarly members of group B of both indole and TMB are hallucinogenic.

As regards the TMB derivatives we have tried to go a little deeper into the reasons why a TMB derivative is turned into a tranquillizing agent or a hallucinogen (Fig. 2).

The compounds on the left are tranquillizing agents and those on the right are hallucinogens. It can be observed that the difference between asarone and mescaline is, first, the position of methoxy group; secondly, the presence of a double bond in the side-chain and thirdly, the presence of unsubstituted amino groups in mescaline. Similar or slight changes in the other compounds can make a compound a tranquillizing or hallucinogenic. Taking advantage of these facts, we have got synthesized in our laboratory a large number of TMB derivatives which, in some respects, resemble asarone, keeping in mind the possibility of striking at a compound which may be a potent tranquillizing agent with minimal side effects. During this process we have screened a large number of compounds which are trimethoxy benzene derivatives. The following groups of compounds were synthesized and pharmacologically screened: (i) trimethoxy phenyl alkyl derivatives, (ii) trimethoxy phenyl benzamides, (iii) trimethoxy phenyl esters, (iv) trimethoxy phenyl anilides, (v) trimethoxy phenyl tetrazoles, and (vi) trimethoxy azlactones.

Alkyl Derivatives

Structure-activity relationship of a number of trimethoxy phenyl alkyl derivatives (Dandiya *et al.*, 1962) has shown that unsaturation in the side-chain is responsible for the central nervous system depressant activity

TABLE 3. S A R OF SOME TRIMETHOXY PHENYL ALKYL DERIVATIVES

S. No.	Alkyl substitution	Position of methoxy groups	S.T.	S.M.A.	C.A.R.	B.P.
1.	$CH_2—CH=CH_2$	3, 4, 5	++	0	0	No effect
2.	$CH=CH—CH_3$	3, 4, 5	0	0	0	↓
3.	$CH_2—CH_2—CH_3$	2, 3, 4	0	0	0	↓
4.	$CH(CH_3)(CH_3)$	2, 3, 4	++	0	0	↓
5.	$CH=CH—CH_3$	2, 3, 4	+	0	0	↓
6.	$C(CH_3)(CH_3)—CH_3$	2, 3, 4	++	0	0	↓
7.	$CH=CH—CH_3$	2, 4, 5	++	0	0	↓

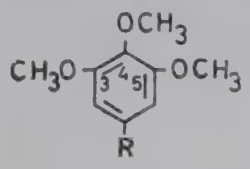
and branching of the side-chain also enhances this activity as seen in compounds 4 and 6 in Table 3.

With alkyl substituents the position of methoxy groups at positions 2, 3, 4 and 2, 4, 5 is of importance in the causation of central nervous system depressant activity.

Benzamides

About 20 benzamides were synthesized in our laboratory [Dandiya *et al.*, 1962; Menon and Dandiya, 1963; Sogani and Dandiya, 1964; Hemnani *et al.*, 1965]. The pharmacological screening of these compounds has revealed an interesting structure-activity relationship. It was found that the most effective substituent is *N*-acetylation, and that di-alkylation decreases the tranquillizing activity while unsaturation brings about an increase in central nervous system depressant activity. The structure-activity of some of the more important trimethoxybenzamides is shown in Table 4. In Table 5 it has been shown that accumulation of methoxy groups brings about central nervous system depressant activity as in compound 3, while dialkyl aminophenyl substitution imparts a central nervous system stimulant and some hallucinogenic activity. It was also inferred that the positions of methoxy groups play an important role in the causation of CNS depressant activity as observed in compound 1 of Table 4 and compounds 2 and 4 of Table 5.

TABLE 4—S. A. R. OF SOME 3, 4, 5-TRIMETHOXYBENZAMIDES



S. No.	R	Potentiation of sleeping time in mice	S.M.A.	C.A.R.	B.P.
1.	$CONH_2$	++	—	—	↓
2.	$CONHCOCH_3$	+++	—	—	↓↓
3.	$CONHCOC_2H_5$	++	—	—	↓
4.	$CON(C_2H_5)_2$	+	0	0	↓
5.	$CH=CHCONH_2$	++	—	0	↓
6.	$CONHC_5H_8$	+	0	0	—
7.	$CONHC_{10}H_7$	+	0	0	—
8.	$CONH\ C_6H_4CH_3$	+	0	0	—
9.	$CONH\ C_6H_4OC_2H_5$	+	0	0	—

Esters, Anilides and Tetrazoles

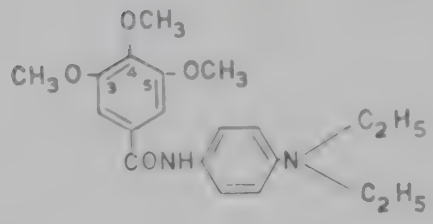
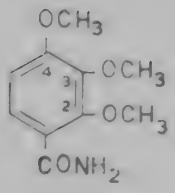
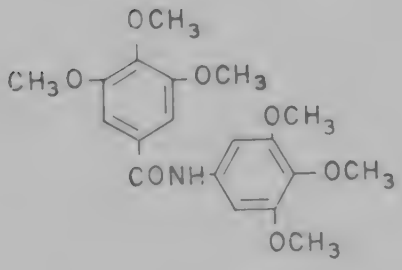
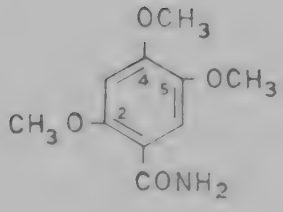
Structure-activity relationship of some 3, 4, 5-trimethoxy esters, anilides and tetrazoles (Dandiya *et al.*, 1962; Sharma, 1965; Sharma and Dandiya, 1965) has shown that esterification is of no importance in central nervous system depressant activity, while anilides show only mild central nervous system depressant activity and tetrazoles are ineffective (Table 6).

Azlactones

Structure-activity relationship of some newer azlactones revealed that azlactone derivatives had CNS depressant activity and substitution of azlactones in 2, 3, 4-trimethoxy phenyl ring makes the compound more active as seen in Table 7 (Sharma *et al.*, 1964).

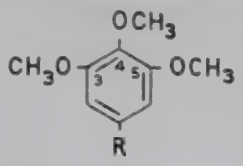
From the foregoing discussion it can be observed that the study of *Acorus calamus* contributed enormously to the possible development of a new series of chemical agents which could be used in psychiatry. This study has provided scientific basis for the use of this plant which has been

TABLE 1: STRUCTURE-ACTIVITY RELATIONSHIP OF SOME [illegible] AND 2, 3, 4-TRIMETHOXYBENZAMIDES

S. No.	Structural formulae	P: of S.T.	S.M.A.	C.A.R.	B.P.
1		0	++	Audiogenic seizure observed	[illegible]
2		+	0	0	[illegible]
3		+-	--	0	[illegible]
4		0	0	[illegible]	[illegible]

employed in mental ailments for the past many years in the Ayurvedic system of medicine. Whether the active principles of *Acorus calamus* will themselves find a respectable basis in modern medicine is still a question, but there appears to be no doubt that a great future exists for the derivatives of these compounds coming up either as pharmacological tools or therapeutic agents or both.

TABLE 6– S. A. R. OF SOME 3, 4, 5-TRIMETHOXY PHENYL ESTERS, ANILIDES AND TETRAZOLE



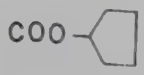
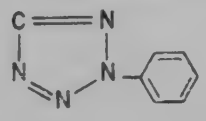
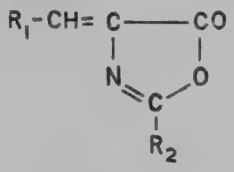
S. No.	R	Sleeping time	S.M.A.	C.A.R.	B.P.
	Esters				
1.	$COOCH_2CH_2N(C_2H_5)_2$	+	0	0	↓
2.		++	0	0	↓
	Anilides				
1.	$NHCOCH_3$	+	—	0	↓
2.	$NHCOC_2H_5$	+	—	0	↓
	Tetrazole				
		0	0	0	0

TABLE 7—S. A. R. OF SOME NEW AZLACTONES



S. No.	R_1	R_2	P of S time in mice	S.M.A.	C.A.R.	B.P.
1.	2, 4, 5-Trimethoxy phenyl	Methyl	+	—	—	0
2.	2, 4, 5-Trimethoxy phenyl	3, 4, 5-Trimethoxy phenyl	+	0	—	0
3.	2, 3, 4-Trimethoxy phenyl	Methyl	++	0	—	↓
4.	Phenyl	3, 4, 5-Trimethoxy phenyl	+	0	—	↓
5.	*p*-Dimethylamino phenyl	3, 4, 5-Trimethoxy phenyl	+	+	—	↓

REFERENCES

1. Eisleitner, F., Mohrs, R. D., Clarke, L. C. de Tom, R. P., *J. org. Chem.*, **23** (1958), 2005.
2. Dandiya, P. C. & Cullumbine, H., *J. Pharmac. exp. Ther.*, **125** (1959), 353.
3. Dandiya, P. C. & Sharma, J. D., *Indian J. med. Res.*, **50** (1962), 46.
4. Dandiya, P. C. & Menon, M. K., *Br. J. Pharmac.*, **20** (1963), 436.
5. Dandiya, P. C. & Menon, M. K., *J. Pharmac. exp. Ther.*, **145** (1964), 42.
6. Dandiya, P. C. & Menon, M. K., *Life Sciences*, **4** (1965), 1635.
7. Dandiya, P. C., Bantle, R. M., Walker, G. C. & Cullumbine, H., *J. Pharm. Pharmac.*, **11** (1959a), 163.
8. Dandiya, P. C., Cullumbine, H. & Sellers, E. A., *J. Pharmac. exp. Ther.*, **126** (1959b), 334.
9. Dandiya, P. C., Sharma, P. K. & Menon, M. K., *Indian J. med. Res.*, **50** (1962), 750.
10. Fabing, H. D. & Hawkins, J. R., *Science*, **123** (1956), 886.
11. Hemnani, K. L., Menon, M. K. & Dandiya, P. C., *Indian J. med. Res.*, (1965) (in press).
12. Hofmann, A., Heim, R., Brack, A., Kobel, A., Frey, A. J., Ott, H. & Troxler, F., *Helv. chim. Acta*, **42** (1959), 1557.
13. Karim, M. A., Linnell, W. H. & Sharp, L. K., *J. Pharm. Pharmac.*, **12** (1960a), 74.
14. Karim, M. A., Linnell, W. H. & Sharp, L. K., *J. Pharm. Pharmac.*, **12** (1960b), 82.
15. Menon, M. K. & Dandiya, P. C., *Indian J. med. Res.*, **51** (1963), 1037.
16. Murphee, H. B., Jenney, E. H. & Pfeiffer, C. C., *Pharmacologist*, **2** (1960), 64.
17. Pennes, H. H. & Hoch, P. H., *Am. J. Psychiat.*, **113** (1957), 887.
18. Sai-Halaszy, A., Brunecker, G. & Szara, S., *Psychiat. Neurol.*, **135** (1958), 285.
19. Schneider, J. A. & Sigg, E. B., *Ann. N. Y. Acad. Sci.*, **66** (1957), 765.
20. Sharma, J. D. & Dandiya, P. C., *Indian J. med. Res.*, **50** (1962), 61.
21. Sharma, P. K., Thesis submitted for Ph.D., Rajasthan University (1965).
22. Sharma, P. K. & Dandiya, P. C., Paper read at the Seventeenth Session of Indian Pharmaceutical Association at Banaras (1965).
23. Sharma, P. K., Menon, M. K. & Dandiya, P. C., *J. pharm. Sci.*, **53** (1964), 1055.
24. Sogani, S. K. & Dandiya, P. C., *J. med. Chem.*, **8** (1965), 139.
25. Szara, S., *Experientia*, **12** (1956), 441.
26. Woolley, D. W. & Shaw, E., *Ann. N. Y. Acad. Sci.*, **66** (1957), 649.

Structure and CNS Activity Relationship in Diazacycloalkane Series

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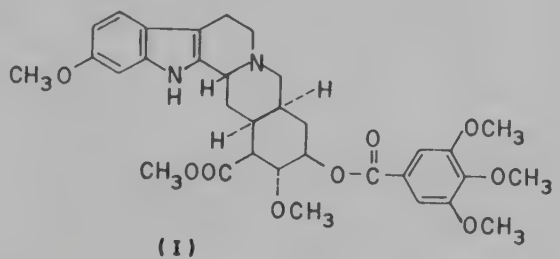
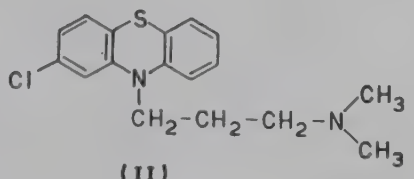
Recent advances in the study and treatment of mental disorders and state of anxiety have opened new vistas of research in synthetic medicinal chemistry. The discovery and therapeutic efficacy of reserpine (I) as a sedative-tranquillizer marked the beginning of a new era in the field of psychotherapeutics. Chlorpromazine (II) was introduced as a drug almost at the same time as reserpine and was found to be a clinically useful tranquillizer.



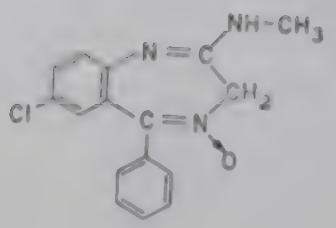
(I)



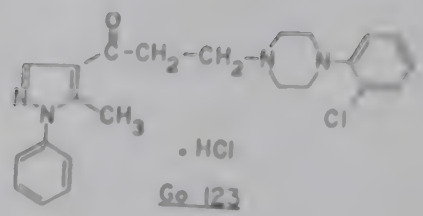
(II)

More recently, a new class of psychopharmacological agents derived from 1,4-benzodiazepines has shown tranquillizing properties. Chlordiazepoxide (III) is a member of this series.

All these compounds exhibit wide structural variations and a great deal of chemical effort has been deployed to modify or improve their CNS



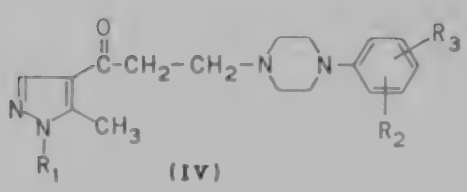
(III)



Go 123

activity. It was, therefore, considered worthwhile to search for new psychopharmacological agents.

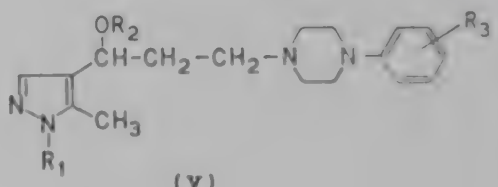
In the early stages of our work, we thought that compounds containing diazacycloalkane rings, e.g. piperazine and homopiperazine may impart CNS depressant activity to organic molecules. Consequently, a large number of compounds containing piperazine moiety have been prepared in this laboratory and submitted for systematic screening for their pharmacological effects on the central nervous system of experimental animals. The present paper describes the synthesis, structure and CNS activity relationship of some of these compounds. It was discovered early in our work that N-[3-(1-phenyl-5-methyl-4-pyrazolyl)-3-oxo-1-propyl] -N'-2-chlorophenyl-piperazine hydrochloride (*Go. 123) had interesting CNS depressant properties. This finding prompted us to synthesize a series of related compounds of the general structures (IV) and (V) to evaluate their CNS depressant effects:



(IV)

R_1 = Aryl

R_2, R_3 = Hydrogen, alkyl, halogen, trifluormethyl, etc.



(V)

R_1 = Aryl

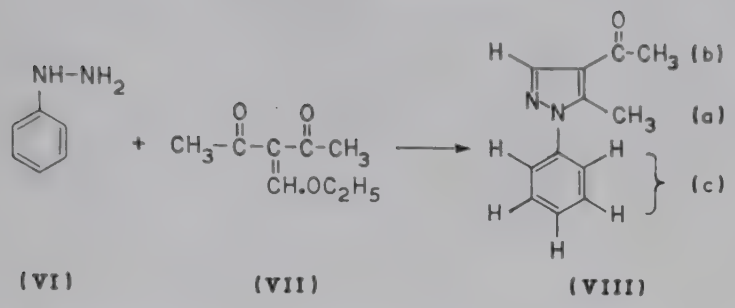
R_2 = Hydrogen, alkyl, acyl

R_3 = Alkyl, alkoxy, halogen, etc.

The synthesis of the starting material, 1-phenyl-4-acetyl-5-methyl pyrazole (VIII) was first carried out by Claisen[1]. While examining the reaction of ethoxymethylene acetylacetone (VII) with phenyl hydrazine (VI), he isolated a crystalline compound, $C_{12}H_{12}N_2O$, m.p. 107-8° C. which formed a methiodide, $C_{13}H_{15}N_2OI$, m.p. 166° C. and an isonitroso derivative, $C_{12}H_{11}N_3O_2$, m.p. 192° C. Based on these observations, the structure (VIII) was assigned to the product.

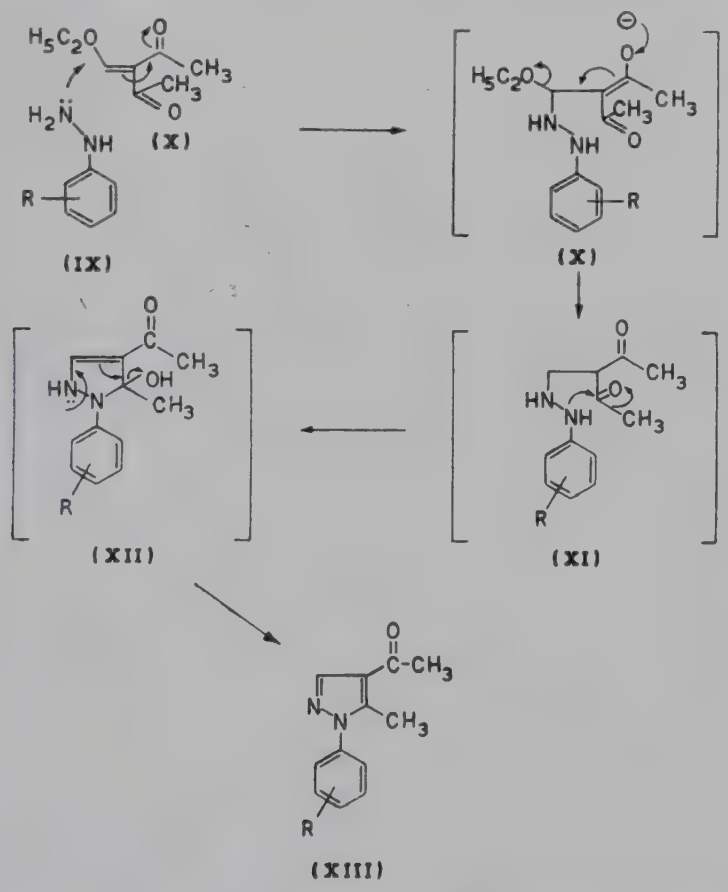
A literature search on the scope of this reaction revealed that this has not been subsequently investigated.

*Go. — is a code initial for compounds synthesized at CIBA Research Centre, Goregaon, Bombay 62, India.



(VI) (VII) (VIII)

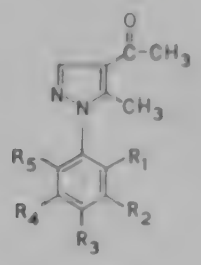
We have confirmed the structure of (VIII) by physicochemical means. The UV-spectrum of this compound shows λ max. at 250 mμ. (ϵ =23,000) in ethanolic solution. The IR spectrum shows carbonyl band at 1660 cm.$^{-1}$ and the NMR spectrum exhibits signals at δ 2·45 (a; 3H; singlet), δ 2·55 (b; 3H; singlet), δ 7·45 (c; 5H; singlet) and δ 7·98 (d; 1H; singlet) which is in complete agreement with the structure (VIII) assigned to this product. The mechanism of the reaction of an aryl hydrazine with ethoxymethylene acetylacetone can be rationalized as follows:



Thus, a number of 1-aryl-4-acetyl-5-methyl pyrazoles (XIV) were prepared by condensing appropriately substituted phenyl hydrazines (IX) with ethoxymethylene acetylacetone. The substituted phenyl hydrazines were prepared by the diazotization of the corresponding anilines followed by

the reduction of the diazonium salts with stannous chloride. The structure of each pyrazole was ascertained by elemental analysis, UV and IR spectra and in several cases by examination of their NMR spectra. Some of the pyrazoles prepared are given in Table 1.

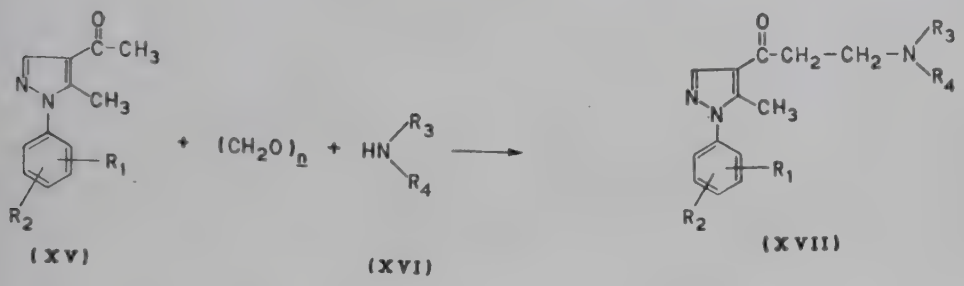
TABLE 1



(XIV)

No.	R_1	R_2	R_3	R_4	R_5
1	H	H	H	H	H
2	H	H	$-NO_2$	H	H
3	H	H	—Br	H	H
4	H	H	$-CH_3$	H	H
5	H	H	—F	H	H
6	Cl	H	H	Cl	H
7	Cl	H	Cl	H	Cl
8	H	Cl	H	H	H
9	H	H	$-OCH_3$	H	H
10	H	CF_3	H	H	H
11	F	H	H	H	H
12	H	F	H	H	H
13	H	H	Cl	H	H
14	H	CH_3	H	H	H
15	CF_3	H	H	H	H
16	$-OCH_3$	H	H	H	H
17	NO_2	H	NO_2	H	H
18	H	H	C_2H_5	H	H
19	H	CF_3	Cl	H	H

Only two compounds described in Table 1 showed some pharmacodynamic properties, e.g. 1-(4-nitrophenyl)-4-acetyl-5-methyl pyrazole (compound 2) had slight sedative activity and weak analgesia was encountered for the corresponding fluoro derivative (compound 5). Other compounds listed in Table 1 had no CNS depressant activity. Some of the pyrazoles were reacted with a number of secondary amines (XVI) under Mannich reaction conditions. For instance, 1-phenyl-4-acetyl-5-methylpyrazole (XV; $R_1=R_2=H$) was reacted as follows to afford the Mannich bases of the general formula (XVII).

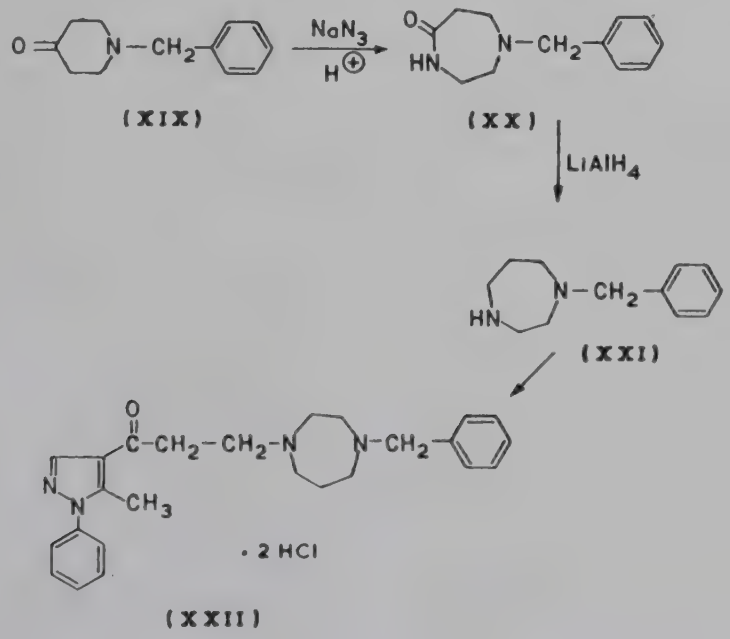


(XV) (XVI) (XVII)

Some of the compounds prepared in this way are given in Table 2.

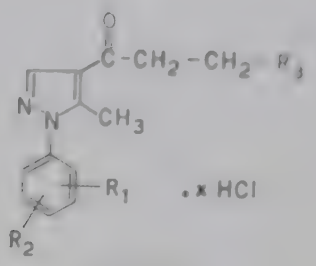
Compound 20 was inactive. Compound 21 exhibited a slight analgesic activity while compounds 22, 24 and 25 showed negligible CNS depressant properties. Other compounds listed in Table 2 as well as Mannich bases derived from N-acyl piperazines had no depressant effect on the central nervous system of experimental animals.

In an attempt to improve upon the activity of compound 24, the corresponding homopiperazine derivative (XXII) was synthesized.

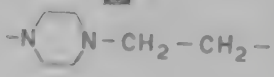
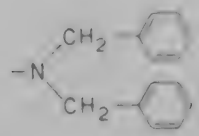
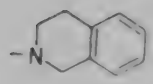
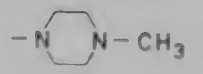
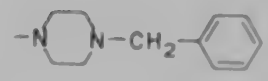


(XIX) (XX) (XXI) (XXII)

TABLE 2



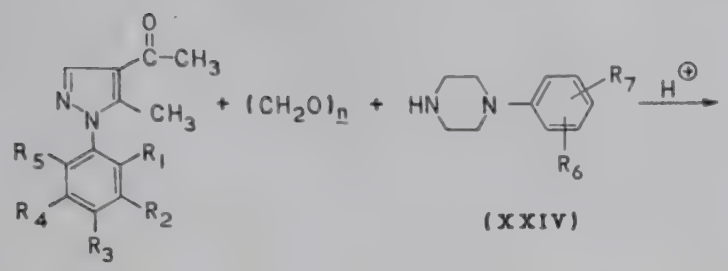
(XVIII)

No.	R_1	R_2	R_3	X
20	H	H		2
21	H	H		1
22	H	H		1
23	2-Cl	5-Cl		2
24	H	H		2
25	H	H		2

This compound (XXII) showed no CNS depressant properties in mice at a dose of 25 mg. kg. *per os*. At higher doses, fatal tonic clonic convulsions were noticed. However, the Mannich products obtained by the reaction of 1-aryl-4-acetyl-5-methyl pyrazoles with paraformaldehyde and N-aryl piperazines under acidic conditions, had significant CNS depressant activity. It is interesting to note that N-aryl piperazines themselves have no CNS depressant activity.

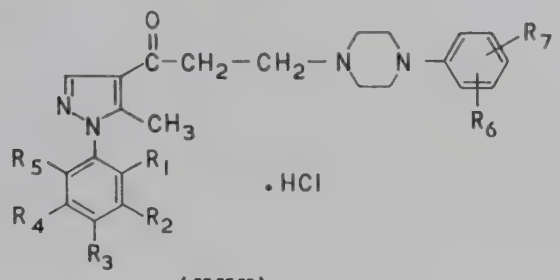
The desired N-aryl piperazines were prepared by the method of Mull *et al.*[2]. Mannich condensation was carried out in ethanol or isopropanol and the resulting products (XXV) were obtained.

Compounds of the type (XXV) are shown in Table 3.



(XXIII) (XXIV)

TABLE 3



(XXV)

No.	R_1	R_2	R_3	R_4	R_5	R_6	R_7
26	H	H	H	H	H	H	4-NO_2
27	H	H	H	H	H	3-CF_3	H
28	H	H	H	H	H	H	4-F
29	H	H	H	H	H	2-CH_3	5-Cl
30	H	H	NO_2	H	H	H	4-F
31	H	H	H	H	H	2-CH_3	5-CH_3
32	H	H	Br	H	H	2-OCH_3	H
33	H	H	H	H	H	2-OCH_3	5-OCH_3
34	H	H	Br	H	H	H	4-F
35	H	H	CH_3	H	H	2-F	H
36	H	H	CH_3	H	H	2-OCH_3	H
37	H	H	NO_2	H	H	2-CH_3	H
38	H	H	H	H	H	2-C_2H_5	H
39	H	H	Br	H	H	2-C_2H_5	H
40	H	H	Br	H	H	2-F	H
41	H	H	Br	H	H	2-Cl	H
42	H	H	F	H	H	2-OCH_3	H
43	H	H	F	H	H	2-Cl	H

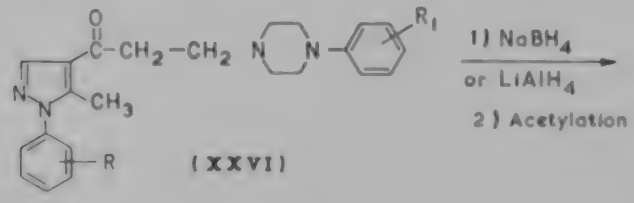
Contd

TABLE 3—*Contd*

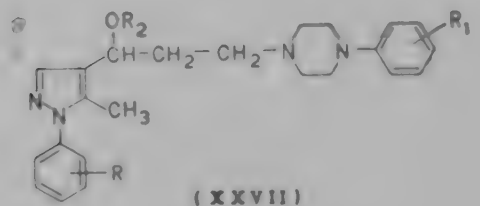
No.	R_1	R_2	R_3	R_4	R_5	R_6	R_7
44	H	H	Br	H	H	3-F	H
45	Cl	H	H	Cl	H	H	4-F
46	Cl	H	Cl	H	Cl	H	4-F
47	H	H	F	H	H	H	4-F
48	F	H	H	H	H	H	4-F
49	H	H	F	H	H	2-F	H
50	F	H	H	H	H	2-F	H
51	H	F	H	H	H	H	4-F
52	H	F	H	H	H	2-Cl	H
53	H	Cl	H	H	H	H	4-F
54	H	Cl	H	H	H	2-Cl	H
55	H	H	Cl	H	H	$2\text{-}OCH_3$	H
56	H	H	Cl	H	H	H	4-F
57	H	H	C_2H_5	H	H	H	4-F
58	H	Cl	H	H	H	$2\text{-}OCH_3$	H

The neuropharmacological assessment of the compounds listed in Table 3 has been done by employing a variety of pharmacological tests[4]. This evaluation indicates that compounds 28, 31, 32, 34, 36, 37, 42, 47, 49, 56, 57 had outstanding CNS depressant activity. Thus, it appears that a halogen substitution in *o*- or *p*-position in aryl pyrazole and or aryl piperazine moieties of the general structure (XXV) is a characteristic feature of the outstanding compounds of this series.

An attempt was made to extend the structure-activity relationship to compounds of the type (XXVII). These compounds have been prepared by $NaBH_4$ or $LiAlH_4$ reduction of the corresponding ketones (XXVI) followed by acetylation. These compounds are listed in Table 4.



(XXVI)

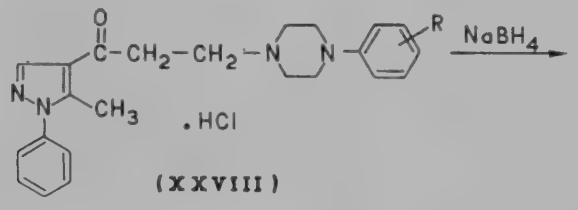


(XXVII)

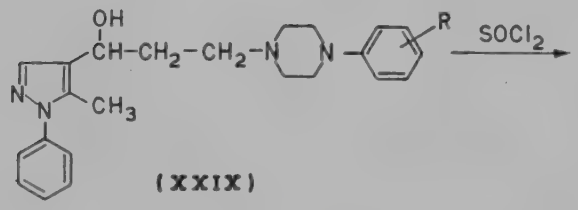
TABLE 4

No.	R	R_1	R_2
59	H	2-Cl	H
60	H	4-Cl	H
61	H	4-F	H
62	4-NO_2	4-F	H
63	4-Br	4-F	H
64	4-Br	4-F	O ‖ —C—CH_3
65	4-Br	2-OCH_3	H

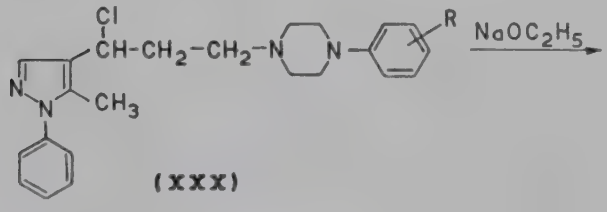
Compounds 59, 63 and 65 had outstanding activity. Compound 59, in addition, had some analgesic properties. The CNS depressant activity of the alcohols was not improved by etherification.



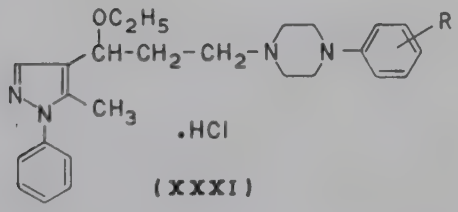
(XXVIII)



(XXIX)

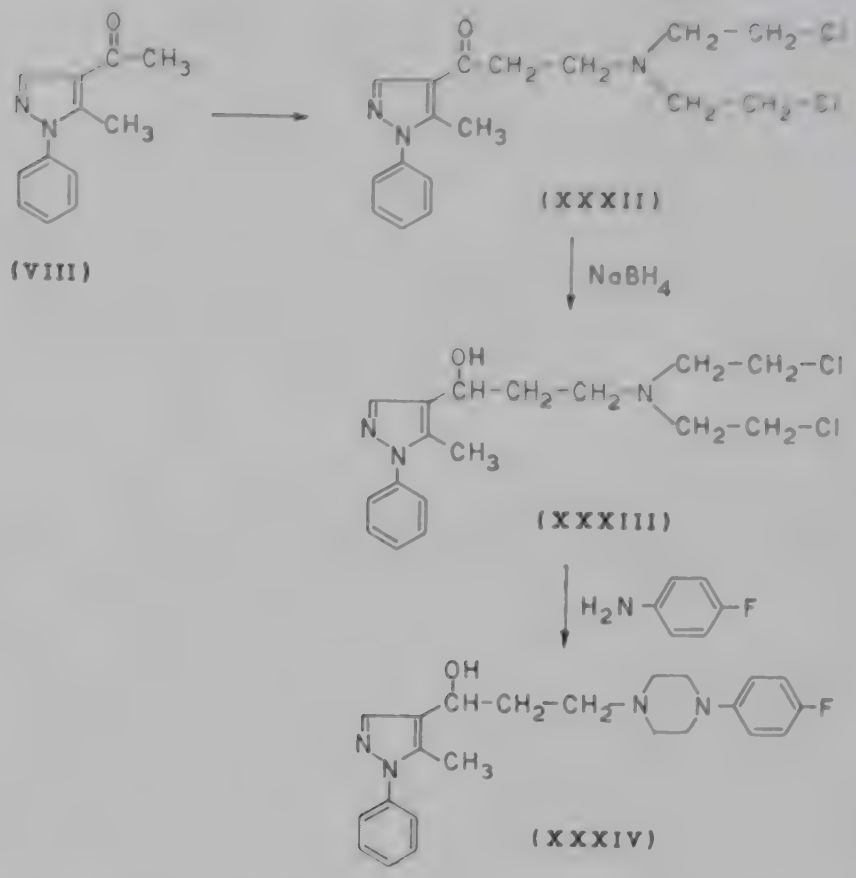


(XXX)

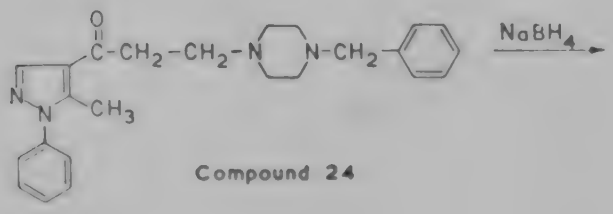


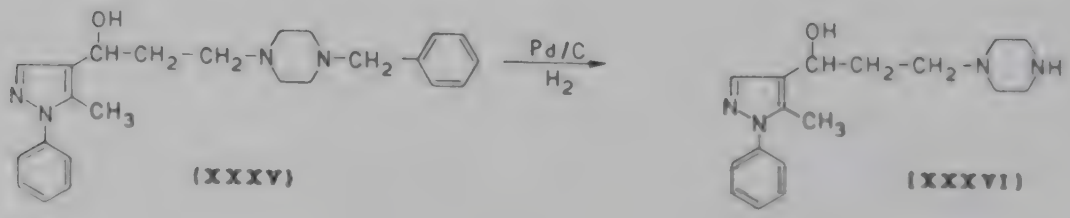
(XXXI)

An alternate synthesis of compound 61 has been carried out by the sequence of reactions (XXXII) → (XXXIII) → (XXXIV).



So far we have discussed the compounds containing N-aryl piperazine and it was of interest to see if an unsubstituted derivative (XXXVI) had any CNS depressant properties. This was prepared as follows :





The compound (XXXVI) is also moderately active. Thus, it appears that the compounds of the present series which possess a diazacycloalkane ring, e.g. piperazine, do possess interesting CNS depressant properties.

ACKNOWLEDGEMENT

The author wishes to express his indebtedness to Dr T. R. Govindachari, CIBA Research Centre, for his interest; Dr George deStevens, CIBA Pharmaceutical Company, Summit, N. J., USA for valuable suggestions; and Dr R. S. Grewal, Dr K. Nagarajan, Dr (Mrs) J. David and Dr P. Rajagopalan for many helpful discussions. The author also thanks Shri S. P. Ghate for technical assistance.

REFERENCES

1. Claisen, L., *Ann.*, **295** (1896), 320.
2. Mull, R. P., Tannenbaum, C., Dapero, M. R., Bernier, M., Yost, W. & deStevens, G., *J. med. Chem.*, **8** (1965), 333.
3. David, J. & Grewal, R. S., personal communications.

Neuropharmacological Actions of Go. 1076*, an Aryl Pyrazole Derivative

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Pharmacological investigations of a series of aryl pyrazole derivatives showed that certain members of the series exhibited hypotonia accompanied by a suppression of locomotor activity. Preliminary screening of Go. 1076 indicated sedative tranquillizing properties. The neuropharmacological investigation of Go. 1076 was extended by a comparison with known central depressant agents such as chlorpromazine and chlordiazepoxide and the results obtained provided the basis for their report.

METHODS AND RESULTS

Behavioral Activity

Effects on Gross Behaviour

The most consistent behavioral effect produced in all the animal species employed, viz. monkeys, dogs, cats, rats and mice, was a suppression of locomotor activity without any evidence of sedation or associated muscular incoordination. This quiescent effect was best observed at a dose level of 25 mg./kg. p.o. in all species, occurring half an hour after administration and lasting for about four to six hours. With higher doses of 50 to 100 mg./kg. p.o., locomotor activity was markedly suppressed, aggressive behaviour was abolished and sleep episodes became increasingly frequent. However, arousability to sound and tactile stimulation remained unimpaired. It was possible to achieve a greater degree of CNS depression with Go. 1076 than with chlordiazepoxide at doses which did not produce muscular weakness or ataxia. In doses which produced equivalent CNS depression,

*N-[3-[1-*p*-Bromophenyl-5-methyl-4-pyrazolyl]-3-oxo-1-propyl]-N'-2-methoxyphenyl-piperazine hydrochloride

chlorpromazine increased the tendency to sleep and produced marked tremors and ataxia.

Go. 1076 produced a marked calming effect in Rhesus monkeys. Under the influence of 25 to 50 mg./kg. p.o. of Go. 1076, aggressive Rhesus monkeys exhibited no hostility or retaliatory attitude when provoked and appeared unresponsive to their environment. Most animals could be picked up without meeting with resistance from them although in the normal state they could not be handled easily. Doses larger than 50 mg./kg. p.o. caused drowziness and ptosis. No ataxia was apparent and the appetite was unimpaired. In our laboratory experiments, chlordiazepoxide in a dose range of 25 to 50 mg./kg. produced ataxia, muscular weakness and reduction of hostility. However, these monkeys could not be handled with ease and retained their awareness of the environment.

Experimentally-induced Aggressive Behaviour

Irritability of septal rats. Brady and Nauta (1953) found that rats with lesions in the septum became extremely vicious within 24 to 48 hr and that this viciousness persisted for 12 days. Their method was employed to compare the effects of Go. 1076 with chlorpromazine and chlordiazepoxide on the irritability of septal rats. Rats with lesions in the cingulate cortex served as controls. The behavioral abnormalities of the lesioned rats were scored according to the method of Randall *et al.* (1960). The mean score following saline injection for rats with septal lesions was 20 while for control cortical lesioned rats, it was nine. The ED_{50} or the dose of the compound reducing the score to half of the pretreatment score was determined graphically. All drugs were administered intraperitoneally. Histological verification of the site of the lesion was done routinely. In the case of rats with cortical lesions, the ED_{50} represents the dose causing hypnosis, whereas in septal rats it represents the dose reducing their irritability to control levels. The results are summarized in Table 1.

Chlordiazepoxide is 5–7 times more potent than Go. 1076 in reducing aggressive behaviour in mice and rats, although such taming was effected at doses which also caused ataxia and muscular weakness. Chlorpromazine was 15 times more potent than Go. 1076 in reducing aggressive behaviour in septal rats. Data in Table 1 show that the ED_{50} for septal rats is lower than that for cortical lesioned rats. Both Go. 1076 and chlordiazepoxide require 2 to 2·5 times the taming dose in order to produce hypnosis in normal rats.

Action on the incidence of fighting among mice following footshock. Fighting episodes were produced in mice by exposing them to mild footshock as described by Tedeschi *et al.* (1959). The ability of the agents to reduce the frequency of the fighting episodes by 50 per cent was taken as the criterion of effectiveness. Drugs were administered orally at different dose levels. The data are summarized in Table 1. Chlordiaz-

TABLE 1—EFFECT OF Go. 1076, CHLORDIAZEPOXIDE AND CHLORPROMAZINE ON THE IRRITABILITY OF RATS AND MICE

Drugs were examined at peak action, generally 2 hr after administration. Doses of 25 rats per dose were employed to estimate ED_{50} in septal rats and rats with cortical lesions.)

Chemical agent	Fighting mice ED_{50} *mg./kg. p.o.*	Antagonism to *d*-amphetamine toxicity ED_{50} *mg./kg. i.p.*	ED_{50} *mg. kg. i.p.*	
			Septal lesion	Cortical lesion
Go. 1076	75 (58·6-96)*	3.7 (2·1-7·00 *	74·5	175·8
Chlordiazepoxide	14·9** (8·5-24·5)*	..	10·2**	21·5
Chlorpromazine	7·2** (2·1-11·4)*	2·00 (0·5-7·7)*	4·9	7·2

*Fiducial limits at P = 0·05
**Loss of grasping reflex at this dose

epoxide is 5 times more effective and chlorpromazine 10 times more effective than Go. 1076 in reducing footshock-induced fighting behaviour, although both reference agents evinced ataxia at the taming dose.

Action on the increased toxicity of *d*-amphetamine in aggregated mice (group excitement). The effect of Go. 1076, chlordiazepoxide and chlorpromazine on amphetamine toxicity in aggregated mice was determined by the method described by Burn and Hobbs (1958). The compounds were given intraperitoneally at four dose levels to groups of 30 mice each and was followed one hour later by 14 mg./kg. of *d*-amphetamine i.p. This dose of amphetamine *per se* produced 100 per cent mortality after 24 hr. Go. 1076 effectively antagonized the amphetamine-induced toxicity. 3·7 mg./kg. i.p. of Go. 1076 protected from death 50 per cent of the aggregated mice whereas chlordiazepoxide even in doses up to 150 mg./kg. i.p. failed to afford protection. The ED_{50} of chlorpromazine was 2·0 mg./kg. and it was as effective as Go. 1076.

Effect on a Learned Response

Conditioned avoidance response (CAR). The apparatus employed was essentially the same as that originally described by Cook and Weidley (1957) and the method followed was that of Swinyard *et al.* (1959). The basic method involved the presentation of a conditioned stimulus (a buzzer) for 10 sec. before the delivery of a shock which was the unconditioned

stimulus. If the conditioned avoidance response (CAR) did not occur after the onset of the buzzer, the shock was presented and an escape response terminated the shock. Many potent tranquillizers block CAR at doses having little effect on the escape response. Groups of 5 to 30 rats were tested with various doses of each drug at the time of peak effect until at least 3 dose levels were established between the zero and 100 per cent response. The inhibition of the CAR was used as a measure of tranquillization. The ED_{50} for Go. 1076 was 13·9 mg./kg. i.p. and it had a specific action on the CAR, without any associated somato-motor effects. In this procedure, complete abolition of avoidance behaviour could not be obtained with chlordiazepoxide. The ED_{50} of chlorpromazine was 4·2 mg./kg. i.p. and it was 3·3 times more effective than Go. 1076 in producing a specific inhibition of the CAR.

Effects on Motor Function

Effects on Spontaneous Motor Activity

The effects on spontaneous motor activity were evaluated quantitatively by the photo-cell method of Dews (1953) as modified by Kinnard and Carr (1957). Three mice were used for each dose level and 15-min. counts were registered at hourly intervals for three hours. Each dose level was repeated several times. Activity counts of untreated mice were obtained in the same manner. The effects on two types of motor activity were determined: (a) voluntary motor activity and (b) amphetamine-induced motor activity. The effects were measured by comparing the recorded counts of the drug-treated groups with control counts. The ED_{50} of Go. 1076 was 6·6 mg./kg. p.o. whereas for chlordiazepoxide and chlorpromazine equivalent effects were obtained at 20 mg./kg. p.o. and 6 mg./kg. p.o., respectively. The ability of Go. 1076 and the reference agents to antagonize the hyperactivity induced by 3 mg./kg. s.c. of *d*-amphetamine was similarly determined. The ED_{50} of Go. 1076 was 8·5 mg./kg. p.o. and that of chlorpromazine was 4·2 mg./kg. p.o. On the other hand, chlordiazepoxide failed to antagonize amphetamine induced hyperactivity in doses up to 80 mg./kg. p.o. (Table 2).

Ataxia

Impairment of motor function and the presence of ataxia were determined by the rota-rod method. Normal mice when placed on a slowly rotating horizontal rod maintain themselves in position for a period of 120 sec. The compounds were administered orally and the AT_{50} (or median effective dose causing ataxia and subsequent inability to negotiate the rota-rod in 50 per cent of the mice) estimated in each case after two hours (Table 2). Go. 1076 reduced the maintenance time on the rod by 50 per cent at a dose level of 78·5 mg./kg. p.o. whereas chlordiazepoxide and chlorpromazine caused similar effects at doses of 16·8 and 9·7 mg./kg. p.o., respectively. Go. 1076 affects spontaneous motor activity at doses well

TABLE 2—EFFECT OF Go. 1076 AND REFERENCE AGENTS ON APOMORPHINE AND AMPHETAMINE-INDUCED MOTOR ACTIVITY AND ROTA-ROD PERFORMANCE IN MICE

Agent	ED_{50} for inhibition of SMA *mg./kg. p.o.*	ED_{50} for antagonism of amphetamine induced activity *mg./kg. p.o.*	AT_{50} * *mg./kg. p.o.*	$\frac{AT_{50}}{ED_{50}SMA}$
Go. 1076	6·6 ± 0·70	8·5 ± 1·4	78·5 ± 9·4	11·90
Chlordiazepoxide	20·0 ± 1·8	..	16·8 ± 1·8	0·84
Chlorpromazine	6·0 ± 0·40	4·2 ± 0·25	9·7 ± 0·8	1·61

*AT_{50} is the median effective dose calculated to cause ataxia in 50 per cent of the mice

below the dose range causing ataxia. In contrast with chlordiazepoxide and chlorpromazine, Go. 1076 shows a wide margin between the dose causing minimal neurotoxicity and the dose inhibiting locomotor activity.

Effects on the Neuromuscular Junction and Spinal Reflexes

Go. 1076 showed no significant effect on the amplitude or tone of contractions of the gastrocnemius muscle induced by stimulation of the sciatic nerve in the anaesthetized cat.

The knee jerk elicited from the anaesthetized cat was examined as an index of monosynaptic reflex activity. Go. 1076 in a dose range of 10–20 mg./kg. i.v. had no marked influence on the knee jerk.

Contractions of the anterior tibial muscle evoked every 30 sec. by stimulation of the afferent tibial nerve for one second with square wave pulses of 0·4 msec. duration, at a frequency of 30 cps. were recorded in chloralose anaesthetized cats. An alternate polysynaptic pathway was thus provided for examining the effects of Go. 1076 on spinal interneuncial circuits. In doses of 0·3 to 3 mg./kg. i.v., Go. 1076 attenuated the polysynaptic flexor reflex by 50 per cent for a period of 3 to 4 hr. Larger doses of 5–10 mg./kg. i.v. completely inhibited polysynaptic reflex activity. Similar effects were obtained with chlordiazepoxide at a dose of 3 mg./kg. i.v., whereas chlorpromazine at an equivalent dose was ineffective. However, in encéphale isolé preparations, Go. 1076 at a dose of 3 mg./kg. i.v. facilitated spinal reflex transmission whereas chlorpromazine and chlordiazepoxide were ineffective.

Potentiation of Pentothal Narcosis in Mice

Go. 1076 was as potent as chlorpromazine in potentiating pentothal-induced sleep in mice. Normal mice, pretreated with 25 mg./kg. i.v. of sodium pentothal, sleep for an average of 180 sec. The end point was the complete recovery of the righting reflex. The ED_{50} of Go. 1076 and of

chlorpromazine was 30 mg./kg. p.o. and 28·7 mg./kg. p.o. respectively. Chlordiazepoxide produced a comparable potentiation of anaesthesia at a dose of 300 mg./kg. p.o.

EEG Effects

Electrocarticograms of encéphale isolé cats indicated the appearance of high voltage, slow waves, of 8 to 10 cps. after administration of 3 to 5 mg. kg. i.v. of Go. 1076. Similar EEG changes were observed with chlordiazepoxide at equivalent doses. EEG activation was elicited in encéphale isolé cats by electrical stimulation of the mesencephalic reticular formation with concentric bipolar electrodes oriented with a Labtronics stereotaxic instrument. The Horsley-Clarke coordinates were: anterior=2, lateral=2 and depth=2. The parameters of stimulation were kept constant and consisted of square waves of 1 msec. duration, frequency 200 cps. and the duration of the stimulus was 3 sec. The voltage was varied from threshold to higher values. Thresholds for the EEG activation ranged from 1 to 2 volts. Go. 1076 in doses of 5–10 mg./kg. i.v. shifted the threshold for activation from 2–8 volts. In the case of chlorpromazine, 10 mg./kg. i.v. raised the activation threshold and reduced the duration of the activation response. On the other hand, no demonstrable depression of the reticular activating system was obtained with chlordiazepoxide even in doses up to 15 mg./kg. i.v. The recruiting response was elicited in three preparations by stimulation of the diffuse thalamic system (n. centrum mediale) at 8–12 cps. with sub-threshold voltage (2–3·5 volts) for 2 sec. Recruitment was depressed by Go. 1076 at a dose level of 20–25 mg./kg. i.v.

Antiemetic Activity

The procedure was based on that of Chen and Ensor (1950) and a total of 5 dogs per dose were used. Drugs were administered orally and apomorphine 0·1 mg./kg. s.c. was given 1 hr later. Drug effects were measured as the 50 per cent reduction in frequency of emesis as compared to previously established control data. The ED_{50} for Go. 1076 was 15·8 mg./kg. p.o. According to Randall *et al.* (1960), only a 34 per cent reduction in the frequency of apomorphine-induced emesis was obtained at 40 mg./kg. p.o. for chlordiazepoxide.

Temperature Regulation

One hour after the administration of 30 and 50 mg./kg. i.p. of Go. 1076 to groups of 10 mice the rectal temperatures recorded were significantly different from control mice given saline (Table 3). The reduction in temperature at these dose levels was significant at $P=0{\cdot}05$.

Anticonvulsant and analgesic action. Go. 1076 did not afford protection against maximal electroshock, pentylenetetrazol and strychnine-induced seizures. No analgesic action was detected.

TABLE 3. EFFECT OF Go. 1076 ON RECTAL TEMPERATURE IN MICE

Agent	Dose mg./kg. i.p.	Average control temp. C.	Av. fall in temp. °C in first 2 hr ± SE.
Go. 1076	10 (10)	37·7	2·1 ± 0·3
	30 (10)	37·4	4·7 ± 0·2
	50 (10)	37·2	6·5 ± 0·2

Figures in parentheses indicate number of animals

TABLE 4—ACUTE TOXICITY DATA ON Go. 1076 AND REFERENCE AGENTS

Agent	LD_{50} in mice		LD_{100} in rabbit mg./kg. i.v.
	mg./kg. p.o.	mg./kg. i.p.	
Go. 1076	1667 ± 285·8	673 ± 99·7	20
Chlordiazepoxide	600 ± 41	228 ± 8	98
Chlorpromazine	392 ± 4	..	18

Effects on catecholamine deposits in the brain. Go. 1076 ha a negligible effect on the catecholamine content of the brain (perso communication from Dr Maitre).

Acute Toxicity

Acute toxicity was examined in CF male mice and the results a expressed in Table 4. The LD_{100} value for acute toxicity in the rabbit w 20 mg./kg. i.v. and the toxicity was characterized by initial spasms, cessa of locomotor activity, paralysis and terminal fatal clonic convulsions. G 1076 has a low order of toxicity and the comparative data are summar in Table 4.

DISCUSSION

The aryl pyrazoles represent a class of compounds which profu CNS depression of a tranquillizing type. Go. 1076, an aryl pyra derivative, shared pharmacological properties common to both chlorp mazine and chlordiazepoxide, but its overall neuropharmacological pro evinced parallelism to chlorpromazine rather than to chlordiazepox

Go. 1076 resembled chlorpromazine in that it antagonized amphetamine toxicity in aggregated mice, abolished a previously-established conditioned avoidance response, lowered body temperature, potentiated pentothal anaesthesia and possessed antiemetic activity. It differed from chlorpromazine and resembled chlordiazepoxide in its ability to block polysynaptic pathways in the spinal cord.

Go. 1076 significantly decreased both spontaneous and amphetamine-induced motor activity to the same degree as chlorpromazine. The reduction of spontaneous motor activity in mice has a high degree of correlation with tranquillizing properties (Gray *et al.*, 1961). A prominent effect of Go. 1076 in mice, as well as in other animals, was the abolition of locomotor activity. However, both chlorpromazine and chlordiazepoxide reduced motor activity in mice at doses which were very close to the ataxic dose, whereas Go. 1076 decreased motor activity at one-twelfth its ataxic dose.

The overall CNS depressant effects of Go. 1076 were more pronounced and of greater duration than chlordiazepoxide. A consistent finding with Go. 1076 was the presence to a marked degree of docility in hostile Rhesus monkeys. Chlordiazepoxide appeared to reduce the hostility of monkeys, but the absolute taming effects were not as prominent as reported by Randall *et al.* (1960). Absence of such taming effects in other animal species, pretreated with chlordiazepoxide, has also been reported by Gray *et al.* (1961). Moreover, when taming implied manageability or handling of the monkeys, both the reference agents showed marked ataxia, whereas no ataxic effects were seen with Go. 1076 in doses up to 100 mg./kg. given orally. In contrast with the effects observed on monkeys, studies on experimentally-induced aggressive behaviour in mice and rats showed that Go. 1076 was less effective than chlordiazepoxide and chlorpromazine. Since the question of species difference in the taming effect of psychotropic drugs is unsettled, the type of behaviour pattern tested may determine the drug action. Jacobsen (1962) has suggested that these complex types of aggression-reactions are released by different mechanisms and are differently influenced by known psychopharmacological agents. It is possible that the net effect on the same function could be achieved by the above-mentioned compounds by different mechanisms.

Go. 1076 produced inhibition of the polysynaptic reflex arc in the intact, anaesthetized cat, but in spinalized cats, facilitatory effects were observed. The evidence obtained from previously mentioned effects of Go. 1076 on spinal reflexes, on thermoregulation, antiemesis and EEG synchronization in spinal cats supports the possibility of a supraspinal target of action. The presence of indifference and unresponsiveness to environmental, auditory and tactile stimuli in monkeys pretreated with Go. 1076 may signify an effect on sensory-induced arousal and this aspect of its action is under consideration.

SUMMARY

The neuropharmacological effects of Go. 1076, an aryl pyrazole derivative, were compared with chlordiazepoxide and chlorpromazine. Go. 1076 and chlorpromazine were approximately equipotent in diminishing spontaneous motor activity in mice, reducing amphetamine toxicity in aggregated mice, lowering body temperature and potentiating pentothal anaesthesia in mice and reducing vomiting in dogs. Chlorpromazine was three times more effective than Go. 1076 in inhibiting the conditioned avoidance response in trained rats. Go. 1076 and chlordiazepoxide blocked the polysynaptic reflex arc at equivalent doses in chloralose anaesthetized cats, whereas chlorpromazine was ineffective. Go. 1076 caused a facilitation of the polysynaptic response after spinalization, but no similar effect was evidenced by either chlorpromazine or chlordiazepoxide.

All the three agents had qualitatively similar depressant behavioral effects on monkeys, although Go. 1076 did not display any associated ataxia. Go. 1076 produced synchronization of the EEG and, like chlorpromazine, it blocked the EEG activation response produced by stimulation of the reticular formation but only at high doses.

REFERENCES

1. Brady, J. V. & Nauta, W. J. H., *J. comp. physiol. Psychol.*, **46** (1953), 339.
2. Burn, J. H. & Hobbs, R., *Arch. int. Pharmacodyn.*, **113** (1958), 290.
3. Chen, G. & Ensor, C. R., *J. Pharmac. exp. Ther.*, **98** (1950), 245.
4. Cook, L. & Weidley, E., *Ann. N. Y. Acad. Sci.*, **66** (1957), 740.
5. Dews, P., *Br. J. Pharmac.*, **8** (1953), 46.
6. Gray, W. D., Oster Berg, A. C. & Rach, C. E., *Arch. int. Pharmacodyn.*, **134** (1961), 198.
7. Jacobsen, E., Proceedings of the First International Pharmacological Meeting (Edited by W. D. M. Paton and P. Lindgren), **8** (1962), 251, Pergamon Press, Oxford.
8. Kinnard, W. J. & Carr, C. J., *J. Pharmac. exp. Ther.*, **121** (1957), 354.
9. Randall, L. O., Schallek, W., Heise, G. A., Keith, E. F. & Bagdon, R. E., *J. Pharmac. exp. Ther.*, **129** (1960), 163.
10. Swinyard, E. A., Wolf, H. H., Fink, G. B. & Goodman, L. S., *J. Pharmac. exp. Ther.*, **126** (1959), 312.
11. Tedeschi, R. E., Tedeschi, D. H., Mucha, A., Cook, L., Mattis, P. A. & Fellows, E. J., *J. Pharmac. exp. Ther.*, **125** (1959), 28.

Some Aspects of Structure-Activity Relationship in Psychotropic Agents of the 1,4-Benzodiazepine Series

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It is very appropriate that this symposium on drugs acting on the central nervous system takes place in India, the homeland of the discovery of one of the first natural psychotherapeutic agents. For centuries, the roots of *Rauwolfia serpentina* were in use in India for treatment of many diseases and the sedative properties of this drug were known for a long time.

In 1931, the first crystalline alkaloids were separated and isolated by Siddiqui and Siddiqui[1] and in the same year Sen Gupta and Bose[2] confirmed the sedative and hypotensive properties of *Rauwolfia serpentina* extracts in animals. This led to increased use of this drug in Indian medicine.

Western medicine was rather slow to study this drug and the popularization of tranquillizers and other psychotherapeutic agents started just over a decade ago. In 1952, Müller, Schlittler and Bein[3] published their studies of *Rauwolfia* extracts resulting in the practical isolation and identification of a pure crystalline alkaloid which they named 'reserpine'. This alkaloid showed a pronounced blood pressure lowering effect and was first introduced as a hypotensive agent. It was soon discovered that it also possessed very useful tranquillizing properties, causing relief from anxiety, which led to its wide use as a psychotherapeutic agent.

Following the introduction of reserpine, the discovery and use of psychotropic drugs progressed with ever increasing pace. In 1953, chlorpromazine was introduced. It is a phenothiazine derivative which was initially investigated as an antihistaminic agent. In addition to a variety of effects on the autonomic nervous system, it was found to have very pronounced

psychotropic properties. The increasing use of these two valuable psychotherapeutic agents stimulated intensive studies in this field. The investigation of various types of compounds led to the synthesis and introduction of meprobamate which found wide use and became extremely popular.

At this point it might be appropriate to try to define the word 'tranquillizer' which I have used a few times. It was coined to describe this new class of psychotropic drugs which I have just discussed. In a very primitive manner, tranquillizers can be defined as psychotherapeutic drugs which possess anxiety-relieving properties at doses which do not cause excessive sedation or sleep. However, they also have an effect on the sleeping pattern by relieving disturbing anxiety and tension, but in normal therapeutic doses, do not possess the strong hypnotic properties characteristic of typical 'sleeping drugs'.

The latest addition to the group of tranquillizers was chlordiazepoxide, a 1,4-benzodiazepine derivative which was discovered in the middle fifties and introduced in 1960.

This compound resulted from exploratory synthetic chemical studies concerned with members of a new class of chemical compounds and of the careful investigation of their pharmacological properties. In the search for drugs possessing effects on the CNS, we studied the almost unexplored class of compounds belonging to the group of quinazoline 3-oxides[4]. After the synthesis of many compounds which were of no great interest, we finally treated one of these quinazoline 3-oxides (Fig. 1) with methylamine and obtained a well-defined crystalline reaction product.

Chemical and physicochemical studies revealed that this substance was not the normal substitution product (II) but a compound formed via an unexpected and novel ring expansion which resulted in the formation of a 1,4-benzodiazepine derivative[5]. This compound (III) showed very interesting psychopharmacological properties[6] in the first screening experiments and

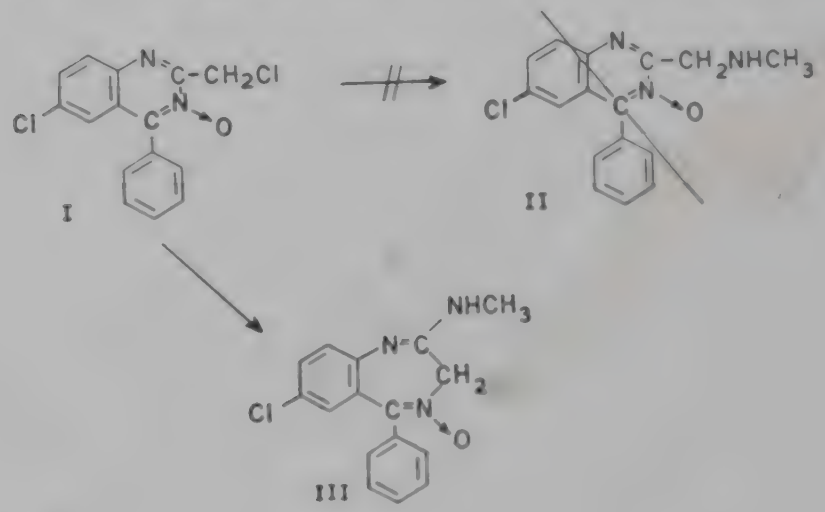


FIG. 1

was ultimately introduced under the trade name of Librium(R) (generic name chlordiazepoxide).

Our primary pharmacological screening for depressant effects on the central nervous system consists of six tests which measure muscle relaxation, taming effects and anticonvulsant properties.

Table 1 shows these tests, the effects which are being measured, literature references and the maximal doses of drug which are used routinely in each of these determinations. The compounds were administered orally and in five tests the doses which caused the effect in 50 per cent of the animals (ED_{50}) were established.

When measuring the muscle relaxation in the cat, the minimal effective dose (MED) was determined by subjective observation.

Many additional properties of our compounds were then determined. The acute toxicity values were established; attempts were made to determine the hypnotic dose, and the effects on blood pressure and on the autonomic nervous system were measured. Compounds which gave interesting screening results were then subjected to a large battery of tests aimed at the exact determination of their psychotropic properties. These tests involved observations on taming effects in monkeys and conditioned avoidance effects in various animal species.

The scope of this paper is, however, limited to discussion of the values obtained in the above six screening tests. No attempt is made to evaluate the differences in the pharmacological spectra, which are quite noticeable in some cases, and only some conclusions are drawn as to the general potency of the compounds under discussion.

Studying the relationship between structure and activity, we found some very interesting regularities which, in many cases, enabled us to predict structures having an increase or decrease in biological activity. However, it may be pointed out that high pharmacological activity is by no means the most important criterion for the clinical value of a drug. Lower activity in some of the tests frequently results in a different pharmacological spectrum which may lead to more useful compounds. Such substances are then tested mainly for their specific activity and are evaluated for example, as muscle relaxants, anticonvulsants or sleep-inducing agents or even as drugs acting in psychotic disorders. Clinical investigators in our laboratory are actively engaged in the study of the value of 1,4-benzodiazepine derivatives for disorders requiring drugs with properties of various types.

After this digression, I would like to return to the main topic, i.e. discussion of the pharmacological properties of various 1,4-benzodiazepine derivatives.

Table 2 gives the data which first sparked our interest in this new compound and shows the comparison of the effects of this product with those of the tranquillizers chlorpromazine, meprobamate and reserpine and also with phenobarbital as a representative hypnotic drug.

TABLE 1—TESTS FOR DEPRESSANT EFFECTS ON THE CNS

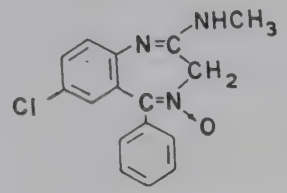
	Inclined screen[1]* (ED_{50})	Foot shock (fighting mice[2]* ED_{50})	Effect in the unanaesthetized cat* (MED)	Pentylene tetrazole (Metrazol(R)) shock[3]† ED_{50}	Maximal electroshock[3,4] (ED_{50})	Minimal electroshock[4]† ED_{50}
Indicative of:	Muscle relaxation	Taming effect	Muscle relaxation	Anticonvulsant	Anticonvulsant	Anticonvulsant
Maximum dose of compound used	500 mg./kg.	100 mg./kg.	Mostly 20 mg./kg. In some cases 50–100 mg./kg.	800 mg./kg.	800 mg./kg.	800 mg./kg.

1. Randall, L. O., Schallek, W., Heise, G. A., Keith, E. F. and Bagdon, R. E., *J. pharm. Ther.*, **129** (1960), 163.
2. Tedeschi, R. E., Tedeschi, D. H., Mucha, A., Cook, L., Mattis, P. A. and Fellows, E. J., *J. Pharmac. exp. Ther.*, **125** (1959), 28.
3. Everett, G. M. and Richards, R. K., *J. Pharmac. exp. Ther.*, **81** (1944), 402.
4. Swinyard, E. A., Brown, W. C. and Goodman, L. S., *J. Pharmac. exp. Ther.*, **106** (1952), 319.

*These tests were carried out by (Mrs) B. Kappell.

†The anticonvulsant activity was determined by R. Banziger and his co-workers to whom we are grateful for the data, used throughout this paper.

TABLE 2

Compound	Incl. Sc.	Footshock fighting mice	Cat	Pentylene tetrazole test	Max.	Min.	Hypnotic HD_{50}	Toxicity LD_{50}
								
Chlordiazepoxide	100	40	2	18	92	150	No effect below toxic dose	p.o. 620
Meprobamate	250	250	100	150	200	167	450	p.o. 1450
Chlorpromazine	17	20	2·5	42	150	600	No effect below toxic dose	i.p. 79
Phenobarbital	120	80	10	75	18	90	87·5	p.o. 242
Reserpine	10*	20*	0·1†	‡	‡	‡	No effect below toxic dose	i.p. 460

*Effect appeared only after *c.* 16 hr and not after one hour as in the other compounds

†Cats became lethargic but did not show the muscle relaxation typical for the other four compounds

‡Does not show anticonvulsant properties resembling those of the other four compounds [*see* Chen, G., Bohner, B., *J. Pharmac. exp. Ther.*, **159** (1965), 559].

In addition to the data indicating the activity in the tests already discussed, Table 2 also includes, for purpose of comparison, data showing the hypnotic dose and toxicity. In our further presentation we shall discuss and compare only the activities in the first six tests since hypnosis was almost never observed except at the toxic dose, and the toxicity was extremely low in every case. The toxicity values varied between 680 and 2900 mg/kg. The extremely high safety margin between the active dose and the lethal dose is characteristic for all compounds belonging to the group of 1,4-benzodiazepines.

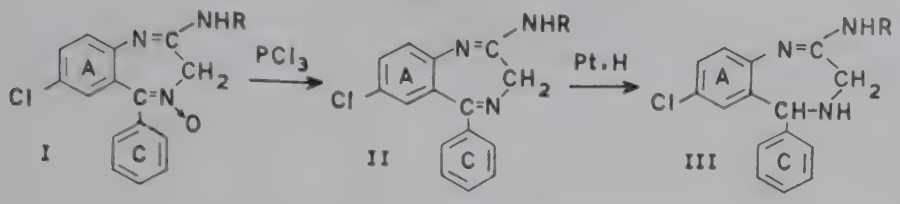
These data showed that chlordiazepoxide had an interesting spectrum of activity resembling, in some respects, each of the four other drugs. In our crucial six tests it was considerably more active than meprobamate; in the first three tests, however, it was less potent than chlorpromazine. It was a potent muscle relaxant and sedative in the mouse and cat and showed taming and strong anticonvulsant properties in mice. Very interesting was the lack of hypnotic properties which differentiated it very significantly from phenobarbital. The complete lack of effects on the autonomic nervous system was also quite remarkable and distinguished it quite characteristically from chlorpromazine and reserpine.

The interesting properties of chlordiazepoxide encouraged us to synthesize a number of very closely related compounds in which only the substituent on the exocyclic amino group was varied. As can be seen from Table 3*, lengthening of the side-chain in compounds of formulae (I), (II) and (III) had a rather unfavourable effect on the activity (compare I with Ib). Removal of the N-oxide oxygen in this series did not increase the activity (compare I with II, Ib with IIb). Hydrogenation of the 4,5-double bond resulted in compounds of formula (III). This modification had a rather negative effect which resulted in a significantly different pharmacological spectrum.

The effects of other variations in compounds of type (I), (II) and (III) (Table 3) were also studied at the beginning of our investigations. These variations were changed in the position and character of substituents in ring A, and also in the introduction of additional substituents in ring C.

*In order to obtain comparable and meaningful figures the entire discussion of pharmacological properties has been based solely on experiments carried out in our laboratories. The figures shown are not identical in every case with our earlier published findings. This is due to the usual variations encountered in all pharmacological determinations of this type; even if the experiments are carried out in the same laboratory and by the same investigators, additional variations are caused by the use of different strains of mice in our later experiments. However, these variations remain within the usual limits of error and, therefore, the conclusions are based only on very pronounced differences which are definitely meaningful. It may be emphasized here that frequently compounds which did not show activity in the usual screening procedure, showed activity when doses higher than the ones shown in the Tables were used.

TABLE 3



Compound	R	Incl. Sc.	FS	Cat	Metrazol	Max.	Min.
I	CH_3	100	40	2	18	92	150
Ia	C_2H_5	225	80		31	67	500
Ib	$(CH_2)_3CH_3$	338	>100	10	50	200	800
II	CH_3	328	100	10	75	75	533
IIb	$(CH_2)_3CH_3$	>500	>100	>20	60	300	>800
III	CH_3	excited conv. 300	>100	>20	>200	66	>200

The synthesis of around 150 related compounds* was the result of these studies.

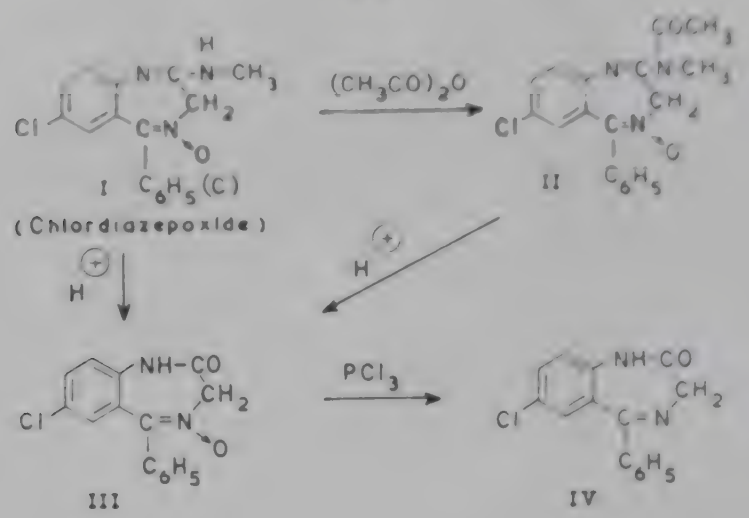
The effect of these variations on the pharmacological properties are not discussed since they parallel in most cases the regularities observed in the study of the 1,4-benzodiazepin-2-ones which is the main topic of this paper.

Further transformations of the chlordiazepoxide molecule showed that the pharmacological properties in the six tests discussed in this paper remained unchanged if the molecule was stripped of many of its characteristic features. Information in Table 4 shows the transformations and their effect on the activity.

It can be seen that the activity was hardly affected and that the rather simple compound (IV) was at least as active as the starting material (I). The high activity of the benzodiazepinone (IV) induced us to search for a simpler preparative route, and for general methods which could be applied to the synthesis of related compounds. These studies ultimately resulted in two methods which proved particularly useful. They are shown in Fig. 2.

*For references consult Sternbach, L. H., Randall, L. O. and Gustafson, S. R., "1, 4-Benzodiazepines (Chlordiazepoxide and Related Compounds)", in Psychopharmacological Agents (Vol. I), M. Gordon, ed., p. 137.

TABLE 4



Compound	Incl. Sc.	FS	Cat	Metrazol	Max.	Min.
I	100	40	2	18	92	150
II	100	20	2	15	150	150
III	75	40	1	6	52	400
IV	75	20	1	6	25	61

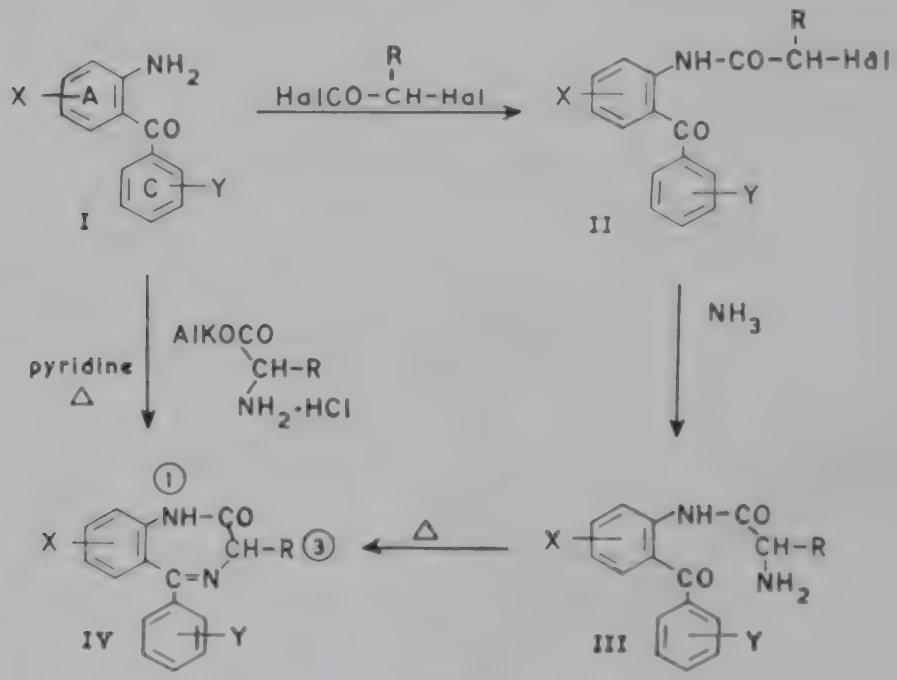


Fig. 2

Sternbach, L. H., Van Fraver, R., Metlesics, W., Reeder, E., Sach, G., Saucy, G. and Stempel, A., *J. org. chem.*, **27** (1962), 3788.

Treatment of the appropriately substituted aminobenzophenone with a haloacetylhalide yielded a compound of type (II) which, on treatment with ammonia, gave the benzodiazepinone (IV) via an open chain intermediate (III). Another extensively used method was the treatment of an amino benzophenone with an amino acid ester hydrochloride in pyridine. The first method generally gave better yields, although it consisted of more steps. The second method facilitated the synthesis of benzodiazepinones bearing substituents at position 3, since many α-amino acids bearing various substituents are commercially available.

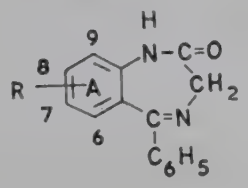
The only factor limiting the use of both of these methods was the accessibility of the appropriate aminobenzophenones. Since many such ketones were known and it was not too difficult to synthesize new ones, the possibility of the preparation of benzodiazepinones with various substituents in rings A and C, was almost unlimited. Additional substituents at position 1 could be introduced readily. Thus several hundred benzodiazepinones were synthesized which enabled us to study rather thoroughly the effect of substituents on the activity. The findings, which seemed most general and meaningful, are presented and illustrated by a few representative examples.

Table 5 illustrates the effect on the activity of substituents in ring A. It was found that substitution of position 7 was of prime importance in the formula shown.

It can be seen that the compound bearing no substituent in ring A showed some activity. This, however, increased very significantly with substitution at position 7. Fluorine had the weakest effect but substituents with

TABLE 5

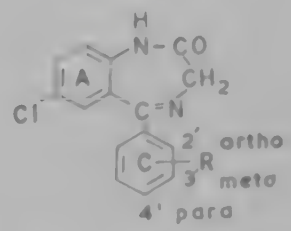
SUBSTITUENTS IN RING A



Compound	R	Incl. Sc.	FS	Cat	Metrazol	Max.	Min.
1		150	100	>30	>800	30	75
2	7-F	150	100	4	>800	23	100
3	7-Cl	75	20	1	6	25	61
4	7-Br	25	20	0·5	1·7	3·7	200
5	7-NO_2	15	5	0·1	0·5	8·4	132
6	7-CF_3	10	10	0·1	1	5	21
7	8-Cl	>500	>100		334	150	800
8	9-NO_2	500	>100		>800	400	>800
9	7-CH_3	>500	>100		175	167	>800

TABLE 6

SUBSTITUENTS IN RING C



Compound	R	Incl. Sc.	FS	Cat	Metrazol	Max.	Mis.
1	H	75	20	1	5·9	25	61
2	2′-F	40	5	0·05	0·08	1·5	50
3	2′-Cl	100	40	0·1	0·4	13	115
4	2′-CH_3	150	100	0·5	7·7	21	183
5	2′-OCH_3	300	100	1	7·5	30	133
6	3′-F	200	40	2	4·2	20	667
7	3′-OCH_3	>500	>100		1·8	102	>800
8	4′-F	>500	>100		>800	50	>800
9	4′-Cl	>500	>100		>800	300	>800

stronger electron-withdrawing properties like chlorine, bromine, the nitro- and trifluoromethyl groups caused high activity. These compounds exhibited strong muscle relaxant and taming properties and were also strong anticonvulsants. Substituents at other positions in ring A had a rather detrimental effect. This is exemplified by the 8-chloro- and 9-nitro derivatives, which were only weak anticonvulsants or muscle relaxants.

Electron releasing groups at position 7 had, in general, a similar unfavourable effect on the activity as is exemplified by the 7-methyl derivative.

The data for muscle relaxation and sedation in the cat are not available in every case. This is due to the fact that this test, being rather time consuming, was not always used in the evaluation of compounds showing low activity in the first two tests.

It is interesting to note that this activity is, with few exceptions, paralleled by the activity against the Metrazol [R] shock. This suggests that the anti-Metrazol [R] activity in this series of compounds indicates muscle relaxant or sedative properties*.

Very interesting was the effect of substitution in ring C which we studied most extensively in the 7-chloro derivative shown in Table 6.

*Chen, G. and Postman, R. (A.M.A. Archives of Neurology and Psychiatry 1952, 498 also consider the activity against Metrazol(R) induced shocks as a measure of sedation

The first compounds to be prepared were those bearing a substituent at the *para* position of ring C. It was quite unexpected, as shown in the last compounds in Table 6, that this change resulted in a highly significant decrease of activity. Despite this rather disappointing result, we decided to synthesize an 'electronically equivalent' compound bearing an *ortho*-substituent in ring C. This compound had chlorine at the *ortho* position and was quite unexpectedly more active than the unsubstituted product. 7-Nitro-, bromo-, cyano-, and trifluoromethyl derivatives showed completely analogous results. This led to an extensive study of the effect of *ortho* substitution in ring C. It was found that in the compounds shown in Table 6 a fluorine at that position had a very strong positive effect whereas other substituents had no significant effect or even decreased the activity. These findings are illustrated by the data given in Table 6. It is interesting to note that it made no difference whether the substituent was electron-releasing or electron-withdrawing.

In compounds of the formula shown, *meta* and *para* substituents had a rather unfavourable effect on the activity. This was most pronounced as mentioned above in compounds bearing a *p*-substituent in the ring C.

The general conclusions as to the effects on the activity of substituents in rings A and C are shown in Fig. 3.

The effect of changes in ring B, the heterocyclic moiety of benzodiazepinones, is now discussed.

The simplest variation was the introduction of substituent at position 1. This was achieved by replacement of the hydrogen with an alkali metal and subsequent treatment with an alkyl halide as shown in Fig. 4.

The effect of a substituent at this position was studied very extensively. It was found in compounds of the formula shown practically without exception that an increase in activity was noted when a methyl group was introduced at that position. Some examples are shown in Table 7.

The last compound mentioned in Table 7 combines many features known to increase activity, namely a nitro group at position 7, the *o*-fluoro substituent in ring C and a methyl group at position 1. This combination resulted in an extremely potent compound, maybe the most active benzo-

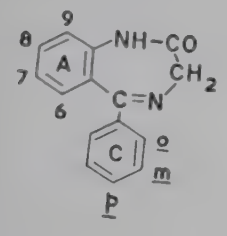


FIG. 3 — EFFECT ON ACTIVITY OF SUBSTITUENTS IN RINGS A AND C

Ring A

Positive: Electron-withdrawing substituents at position 7 (Halogen, CF_3, NO_2, CN) ; Negative : Electron-withdrawing substituents at positions 8 and 9. Electron-releasing substituents at position 7.

Ring C

Positive : Fluorine and chlorine at *ortho* position; Negative: Any substituent at *ortho* position, except fluorine and chlorine. Any substituent at *meta* or *para* position.

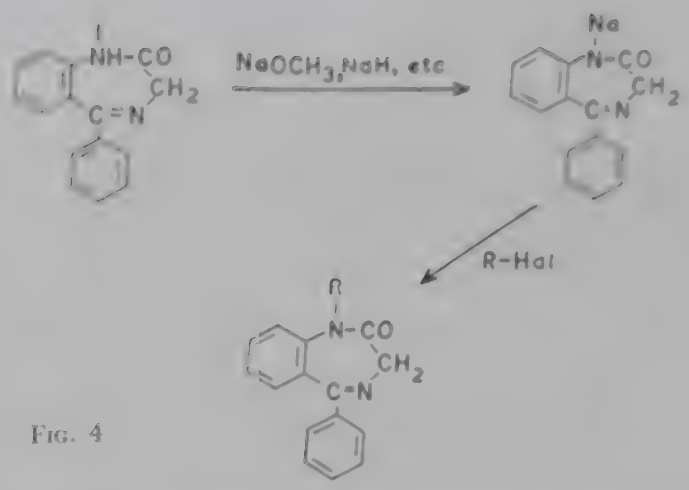
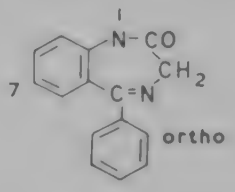


FIG. 4

TABLE 7—EFFECT OF METHYL GROUP AT POSITION 1



COMPOUND	1	7	0	INCL. SC.	FS	CAT.	METRAZOL	MAX.	MIN.
1		Cl		75	20	1	5·9	25	61
1a	CH_3	Cl		30	10	0·2	1·4	6·4	64
2		NO_2		15	5	0·2	0·5	8·4	132
2a	CH_3	NO_2		10	2·5	0·1	0·6	1·5	190
3		$N(CH_3)_2$		>500	>100	10	42	367	667
3a	CH_3	$N(CH_3)_2$		150	40	1	5·9	92	333
4		CN		75	40	0·5	1·3	34	675
4a	CH_3	CN		20	5	0·5	1·2	14	200
5		NO_2	F	4	2	0·05	0·96	20	200
5a	CH_3	NO_2	F	1	0·8	0·02	0·12	12	345

diazepinone derivative known at present. It is interesting to note that compound 3 bearing an electron-releasing substituent at position 7 became quite active after methylation.

It might be appropriate to mention at this point that the effects observed in the benzodiazepine series were generally additive. Products possessing

more than one of the features known to enhance the activity were almost invariably more active than the compounds bearing only one of those characteristics. On the other hand, it was also found that changes which had a detrimental effect on activity exerted the same influence on very potent and on less potent compounds.

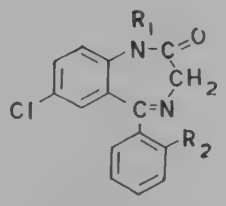
It may be re-emphasized that the highest potency in the tests discussed here is by no means the best criterion for the clinical value of a drug and that lower activity results, in many cases, in products possessing a more interesting and valuable pharmacological and clinical spectrum.

The effect of other substituents at position 1 was studied using the readily accessible 7-chloro derivatives. It was found that heavier or bulkier substituents in the compounds tested generally had a detrimental effect on the activity as could be seen from Table 8.

A pronounced difference in activity between the isomeric compounds bearing a neopentyl or isopentyl substituent at position 1 was also observed. The isopentyl derivative is significantly less potent, at least in the first three tests, than the more compact neopentyl isomer.

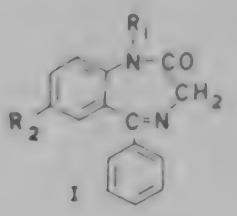
In some compounds some changes in the pharmacological spectrum were also noticed. One of the most interesting compounds in that respect is the methylamidoacetyl derivative (5) which is equipotent to the methyl derivative diazepam (1), in the anticonvulsant tests, whereas its sedative and muscle relaxant properties are 10 to 100 times weaker.

TABLE 8

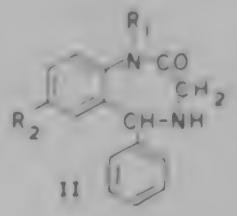


COMPOUND	R_1	R_2	INCL. SC.	FS	CAT	METRAZOL	MAX.	MIN.
1	CH_3	H	30	10	0·2	1·4	6·4	64
2	C_2H_5	H	75	20	1	4	8·3	600
3	$CH_2C(CH_3)_3$	H	160	40	2	22	600	>800
4	$(CH_2)_2CH(CH_3)_2$	H	500	100	20	3·5	100	>800
5	$CH_2CONHCH_3$	H	450	80	20	0·5	15	85
6	CH_3	Cl	40	10	0·1	0·42	15	83
7	NH_2	Cl	250	20	0·5	0·42	20·8	266
8	$(CH_2)_2N(C_2H_5)_2$	Cl	475	100	10	22	150	440

TABLE 9



I



II

Type	R_1	R_2	Incl. Sc.	FS	Cat	Metrazol	Max.	Min.
I	H	Cl	75	20	1	5·9	25	61
II	H	Cl	300	40	10	6·8	40	200
I	CH_3	Cl	30	10	0·2	1·37	6·4	64
II	CH_3	Cl	200	60	4	15	12·5	300
I	H	CF_3	10	10	0.1	1·3	4·7	21
II	H	CF_3	100	40	2	18	43	100

In addition to the effects caused by methylation, it was found that two types of changes in ring B had a profound effect on the activity of 1,4-benzodiazepine derivatives of the formula (I) in Table 9. They were quite general in nature. One was the hydrogenation of the double bond in the 4,5 positions. Without exception, this transformation caused a significant decrease in activity as demonstrated by the examples in Table 9.

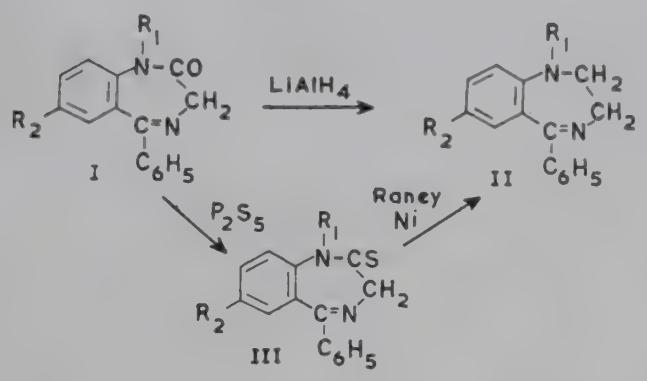
The other transformation was the removal of the carbonyl oxygen at position 2. Replacement of the carbonyl group by a CH_2 group in compounds of formula (I) (Table 10) caused a considerable decrease in activity, and replacement by a CS decreased the activity even more. The methods used for these transformations are given in Table 10, which illustrate the structure-activity relationship of these three types of compounds. In addition, one can see that the '1-methyl rule' also retains its validity in this case.

The illustrated compounds bearing a methyl group at position 1 are more active than the corresponding unsubstituted derivatives; larger substituents cause decrease in activity. The effects are additive as already discussed. The '4,5-dihydro rule' also applies to this group of compounds. Hydrogenation of the double bond at that position generally results in decreased activity.

The most significant findings concerning changes in ring B of 5-phenylbenzodiazepines are summarized in Fig. 5.

The pharmacological study of benzodiazepin-2-ones bearing a thienyl group, a methyl group, a cyclohexyl group or an *a*-pyridyl group at position

TABLE 10



Type	R_1	R_2	Incl. Sc.	FS	Cat	Metrazol	Max.	Min.
I	H	Cl	75	20	1	5·9	25	61
II	H	Cl	90	10	20	4	19	533
III	H	Cl	400	>100		40	800	800
I	CH_3	Cl	30	10	0·2	1·37	6·4	64
II	CH_3	Cl	150	20	2·0	6·7	37·6	283
III	CH_3	Cl	225	60	2	9·2	123	667
I	H	CF_3	10	10	0·1	1·3	4·7	21
II	H	CF_3	50	40	>50	3·7	30	266
I	CH_3	NO_2	10	2·5	0·1	0·5	0·8	133
II	CH_3	NO_2	>500	80	50	4·9	400	800
II	CH_2CH_2 $N(C_2H_5)_2$	Cl	200	100	>20	17	150	>400

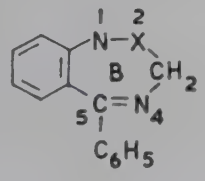


FIG. 5 — EFFECT ON ACTIVITY OF CHANGES IN RING B OF 5-PHENYLBENZODIAZEPINES

A. Position 1 : Optimal activity with a methyl substituent. Less active with hydrogen or substituent bulkier than methyl

B. Position 2 : Optimal : CO
Less active: CH_2
Least active: CS

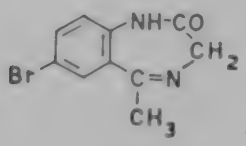
C. Positions 4 and 5 : Hydrogenation of double bond leads to considerable decrease in activity.

5 instead of the phenyl group, provided rather interesting results. Most of the compounds with the exception of those which contain an α-pyridyl group showed low activity as muscle relaxants but seemed to retain specific anticonvulsant properties as given in Table 11.

Quite baffling was the decrease in activity noticed when the 5-phenyl moiety of the illustrated benzodiazepin-2-one is replaced by the isomeric thiophene or the 5-cyclohexyl moiety. These findings were particularly surprising since antihistamines and anticholinergic agents in which a phenyl is replaced by a thienyl or a cyclohexyl group are generally highly active.

In contrast with the above findings, we observed that benzodiazepin-2-ones bearing an α-pyridyl group as a substituent at position 5 were highly active as shown in Table 12.

TABLE 11



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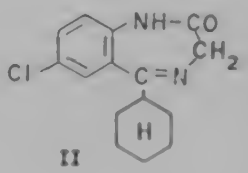
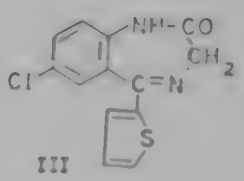


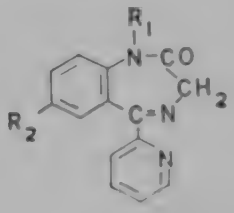
II



III

Compound	Incl. Sc.	FS	Metrazol	Max.	Min.
I	>500	>100	>800	100	300
II	400	>100	150	334	>800
III	>500	>100	100	150	>800

TABLE 12



Compound	R_1	R_2	Incl. Sc.	FS	Cat	Metrazol	Max.	Min.
1	H	Cl	75	10	0·5	0·5	14	177
2	H	Br	30	10	0·2	0·7	34	122
3	$(CH_2)_3N(CH_3)_2$	Br	300	>100	5	84	667	>400
4	H	CF_3	10	10	0·1	1	12	200

During almost a decade of close cooperation between our chemists and pharmacologists, a large body of data relating to the structures of 1,4-benzodiazepine derivatives and their pharmacological activities has been accumulated. It would, therefore, appear to be a simpler task to synthesize further compounds in this series, having predictable psychopharmacological properties. It was, however, observed that the pharmacological spectra of these compounds show in many cases unpredictable variations. This leads us to the expectation that further research in this area will result in the discovery of new drugs which will be highly specific for a wide variety of clinical applications and will continue to show the desirable lack of toxicity which has characterized 1,4-benzodiazepine derivatives todate.

REFERENCES

1. SIDDIQUI, S. & SIDDIQUI, R. H., *J. Indian chem. Soc.*, **8** (1931), 667.
2. SEN, G. & BOSE, K. C., *Indian med. wld*, **2** (1931), 194.
3. MÜLLER, J. M., SCHLITTLER, E. & BEIN, H. J., *Experientia*, **8** (1952), 338.
4. STERNBACH, L. H., KAISER, S. & REEDER, E., *J. Am. chem. Soc.*, **82** (1960), 475.
5. STERNBACH, L. H. & REEDER, E., *J. org. Chem.*, **26** (1961), 1111.
6. RANDALL, L. O., SCHALLEK, W., HEISE, G. A., KEITH, E. F. & BAGDON, R. E., *J. Pharmac. exp. Ther.*, **129** (1960), 163; RANDALL, L. O., Diseases of the Nervous System, Suppl. **XXII**(7), (1961).

A Restatement of Criteria for Evaluation of Centrally Acting Muscle Relaxants with Special Reference to Orphenadrine

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Pharmacological Approaches to the Control of Muscle Spasm

Muscle spasm has been defined as an abnormal involuntary contraction of skeletal muscle (Buller, 1961). The relief of muscle spasm through drug treatment has constituted a major challenge to the pharmacologist. Essentially, three main avenues of approach can be discerned.

The oldest approach made use of sedation. Since muscle spasm is reduced during sleep or drowsiness, drugs were used to induce these states of subdued consciousness and thereby diminish or eliminate the spasm in an indirect manner. The patient thus relieved was, however, more or less incapacitated at the same time. The limitations of such treatment are obvious.

The second therapeutic approach came with the advent of agents such as curare capable of interrupting neuromuscular transmission but devoid of sedative action. It was then hoped to abolish the exaggerated muscle activity underlying spasm without impairing normal motor performance. Disappointingly though, the margin between relaxing and paralysing doses of neuromuscular blocking drugs turned out to be very narrow and respiratory depression or even arrest was frequently encountered. Under these circumstances, a highly predictable dose-effect relationship was an indispensable requirement for their use. It was only partially fulfilled by the intravenous or intramuscular route while other routes of administration were impracticable because of the exceedingly poor and erratic absorption of these compounds due to their quaternary structure. Hence, their use remained restricted to the management of a few drastic motor disturbances

such as tetanus where the patient is under constant supervision of the hospital staff and can be given artificial respiration, should the need occur. The prospects for ever finding a neuromuscular blocking drug, which might be sufficiently selective and predictable in action to enable its widespread use in the treatment of muscle spasm, are slim. The process of neuromuscular transmission in respiratory muscles is fundamentally the same as that in other skeletal muscles of the body; hence, a selective interference with the latter is unlikely to be ever achieved. In considering the physiological basis for a pharmacological intervention at the skeletal nerve-muscle junction, it must also be emphasized that we are confronted with events which take place within the 'final common pathway' of motor innervation, the α-motoneuron. The volleys of nerve impulses which advance here toward the last synaptic relay between their initiation at some point in the central nervous system and the effector organ, the skeletal muscle fibre, exhibit a certain finality as a result of the extensive processing which they had undergone at various levels of the central nervous system. It is unlikely that any further substantial modification or rectification of pathological patterns of innervation can be achieved on this terminal leg of their journey. Nevertheless, recent research has revealed that the neuromuscular junction does not merely transmit nerve messages, as one had been inclined to assume, but possesses properties which would seem to permit integrative processes to occur even at this last possible point of intervention (Katz, 1962). It is tempting to speculate whether these properties of the neuromuscular synapse could ever be expanded by drug action as to measure up to the formidable task of halting the invasion of abnormal impulse activity descending from afflicted nerve centres.

The foregoing discussion of peripheral mechanisms, though beyond the immediate scope of this topic, intends to demonstrate that correction of abnormal motor innervation is unlikely once the descending nerve volleys have entered the peripheral neuron. It is within the central nervous system where such corrective measures should be applied in order to have a better chance to succeed. The tools of this third pharmacological approach to the control of muscle spasm are the centrally acting muscle relaxants which constitute the main theme of this investigation. Drugs effective in Parkinson's disease and those with antiepileptic properties make up separate groups not to be considered within this context; the former, however, do partially overlap with muscle relaxants proper.

Delineation of Centrally Acting Skeletal Muscle Relaxants

It is easy to define this class of agents from a clinical point of view: a centrally acting muscle relaxant drug should relieve skeletal muscle spasm of neurogenic or traumatic-inflammatory origin without impairing consciousness and voluntary motor performance (for a more elaborate clinical description of the 'ideal muscle relaxant', *see* Schwab, 1964). Accordingly,

such a drug has to be free of those major side effects which attend the muscle relaxation obtained with sedative-hypnotic and neuromuscular blocking agents, respectively. Again, it is easy to state the pharmacological correlates of these effects: in doses adequate to produce muscle relaxation, the drug must not depress the ascending reticular formation, the substrate for arousal and wakefulness (Moruzzi and Magoun, 1949), on the one hand, and it must not affect neuromuscular transmission on the other. It will, however, be far from easy to delineate the neuropharmacological correlates of the main action of these drugs, the clinical muscle relaxation. It is in this area where a revision of existing concepts and criteria will be required, not only in the light of recent experimental findings with orphenadrine (Ginzel, 1965 and 1966), a drug possessing clinical muscle relaxant properties, but also from fundamental neurophysiological considerations which appear to have not been heeded in the past.

What then are the criteria that have been used for the pharmacological identification of centrally acting muscle relaxant drugs? Mephenesin, the classic prototype in this category (Berger, 1949), emerged as a new type of relaxant drug by virtue of three main actions. First, the drug produced an ascending flaccid motor paralysis with the convenient end-point of abolition of the righting reflex; this paralysis was not caused by a neuromuscular blockade and was not associated with anaesthesia or respiratory depression (Berger and Bradley, 1946; Berger, 1947). Secondly, the drug blocked polysynaptic spinal reflexes (del Castillo, 1948). Thirdly, it antagonized strychnine convulsions (Berger, 1947). It can be generally stated that this triad of properties (Berger, 1959; Berger *et al.*, 1960), two of which can be tested in mice, has set the stage for the search of muscle relaxant agents between 1949 and today, to a degree which is difficult to understand.

Table 1 reflects this situation. The more elaborate probings into the nature of the relaxant action of mephenesin, such as the elegant studies on extrapyramidal motor systems initiated by Henneman *et al.* (1949), Henneman and Scherrer (1949) and Kaada (1950) were not employed in the majority of investigations of new centrally acting muscle relaxant drugs introduced after mephenesin (Domino, 1964). These former studies revealed the unique action of mephenesin on systems concerned with the control of motor performance through facilitatory and inhibitory pathways which form the substrate of such phenomena as spasticity, rigidity, etc. (Niemer and Magoun, 1947; Lindsley *et al.*, 1949), and have, thus, provided a measure of insight into the clinical effectiveness of the drug against muscle spasm. This cannot be said of the triad of effects described earlier. Though persuasively simple and expedient in use they do not appear to be representative of the clinical activity sought and their adoption for the extensive screening programmes that followed during a period of more than 15 years may well have been the cause for the failure of finding a better mephenesin-type relaxant. The recent emergence of orphenadrine citrate (Norflex®) as

one of the most potent clinical muscle relaxant drugs in current use (Literature Review, 1964) which failed to paralyse mice, abolish polysynaptic reflexes and control strychnine convulsions (Cronheim, 1964) has prompted this inquiry into the pharmacological methodology for detecting central muscle relaxant properties.

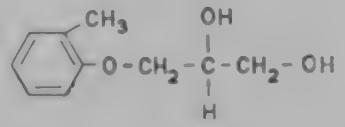
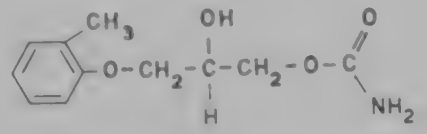
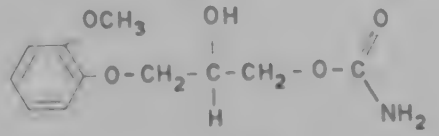
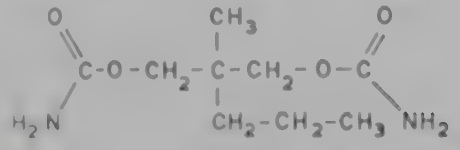
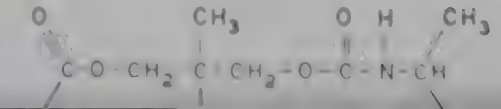
Effect of Orphenadrine on Motor Systems

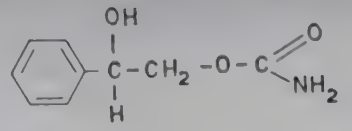
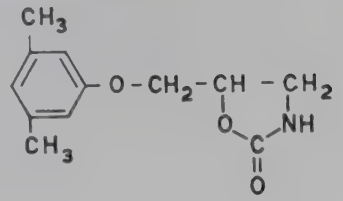
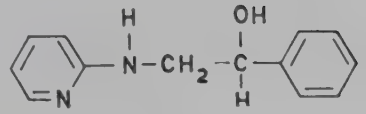
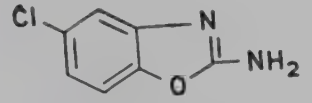
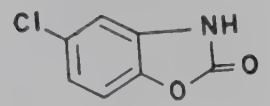
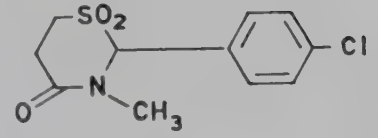
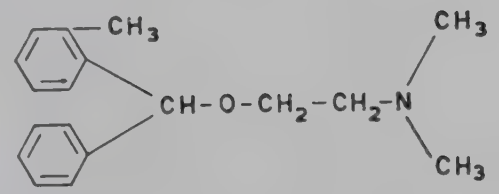
It may be appropriate to describe first the recent pharmacological findings with orphenadrine (Ginzel, 1965 and 1966) which relate to the clinical effectiveness of the drug in muscle spasm. The experimental basis for the efficacy of orphenadrine in Parkinson's disease will not be discussed in this context (de Maar, 1956). The action of orphenadrine on facilitatory and inhibitory motor systems was studied in cats under chloralose, in a few experiments under pentobarbital, anaesthesia and in decerebrate (intercollicular) and spinal preparations. Bipolar electrodes were placed stereotactically in one of the following areas: lateral and rostral region of the reticular formation for facilitation, ventromedial bulbar reticular formation, anterior lobe of the cerebellum, caudate nucleus and pericruciate cortex for inhibition of the patellar reflex; the latter was elicited at intervals of approximately 10 sec. by an automatic hammer and was recorded semi-isotonically with a displacement transducer. Facilitation and inhibition of the patellar reflex was produced also by contra- and ipsilateral sciatic nerve stimulation. In a few cats the linguomandibular reflex was elicited by stimulation of the tongue (King and Unna, 1954). Artificial respiration was maintained throughout, blood pressure stabilization was employed in a few instances. All injections were made intravenously. It is imperative to use 2 or 3 different submaximal voltages for stimulation so as to obtain graded responses which can be plotted as stimulus-response curves. In this way, drug action can be judged more accurately than if only one voltage is employed (Abdulian *et al.*, 1960).

In this experimental setting, orphenadrine was found to block significantly reticular and spinal facilitation of the knee jerk in a dose which, when given to a normal unrestrained cat, did not cause any gross behavioral changes, motor weakness or sedation. Further, the drug abolished the enhanced extensor tonus and the clonus in decerebrate cats. Orphenadrine surpassed mephenesin in potency by 5 times with respect to the blocking action on facilitation and by 10 times with respect to the alleviation of decerebrate rigidity. Moreover, its action lasted substantially longer than that of mephenesin. In contrast with mephenesin, however, orphenadrine did not block any of the inhibitions elicited from various levels of the central nervous system except when given in near toxic doses. This differential action of orphenadrine could be most strikingly demonstrated in experiments in which stimulation of one point elicited reciprocal effects, such as facilitation of the knee jerk associated with inhibition of the linguomandibular

TABLE 1—COMPARATIVE ACTIVITIES OF SOME SKELETAL MUSCLE RELAXANTS

[The effects in 'routine tests' are simply indicated as present (+) or absent (−) since no single study has compared these drugs quantitatively from Creaghead, 1964). Relative potencies of blocking effects on decerebrate rigidity (Berger *et al.*, 1960) and reticular facilitation of some (ork present study). Approximation of clinical efficacy on the basis of maximum recommended daily oral dose.]

Generic name (Trade Name Manufacturer)	Structural formula	Routine tests: Motor paralysis	Routine tests: Blockade of polysynaptic reflexes	Routine tests: Strychnine antagonism	Relief of decerebrate rigidity/relative potency (per cent)	Blockade of reticular facilitation of patellar Reflex/relative potency (per cent)		Relative reciprocal value of maximum clinical dose (per cent
MEPHENESIN (Tolserol-Squibb)					i.v. 10*	i.v. 20	i.p. 50	
MEPHENESIN CARBAMATE (Tolseram-Squibb)		−	−	−				
METHOCARBAMOL (Robaxin-Robins)						[illegible]	[illegible]	[illegible]
MEPROBAMATE (Miltown-Wallace) (Equanil-Wyeth)		+	+	+	10*		15	10
CARISOPRODOL (Soma-Wallace)					[illegible]*	[illegible]	[illegible]	[illegible]

STYRAMATE (Sinaxar-Armour)		+	+	+				
METAXALONE (Skelaxin-Robins)		+	–	–			10	6
PHENYRAMIDOL (Analexin-Irwin, Neisler)		+	–	–			5	3
ZOXAZOLAMINE (Flexin†-McNeil)		–	–	–			50	
CHLORZOXAZONE (Paraflex-McNeil)		–	–	–			20	10
CHLORMEZANONE (Trancopal-Winthrop)		–	–	–			15	40
ORPHENADRINE (Norflex-Riker)		–	–	–	100	100	100	100

†Now in disuse

reflex (King *et al.*, 1952 and 1953) and in which only the former was abolished by the drug. Thus, it appears that orphenadrine is more selective than mephenesin by blocking preferentially those neuronal circuits whose hyperactivity may be implicated in spasticity and other states of hypertonia (Landau *et al.*, 1949). Orphenadrine did not diminish the monosynaptic knee jerk or the polysynaptic linguomandibular reflex.

This experimental evidence offers an explanation for the clinical effectiveness of orphenadrine regardless of its failure to depress righting, polysynaptic reflexes and strychnine convulsions. It will now be examined how valid these three criteria are in characterizing central muscle relaxant activity.

Motor Paralysis

The abolition of righting reflex in the mouse is the end-point by which voluntary motor paralysis can be expressed quantitatively. It can have various causes such as anaesthesia, coma and neuromuscular blockade. The cause of the flaccid motor paralysis produced by mephenesin is still not understood (Longo, 1961). This paralysis differs from the paralysis of neuromuscular blocking drugs in that it starts in the hindquarters of the animal and ascends towards the head, whereas the paralysing action of neuromuscular blocking agents progresses in the opposite direction. Further, it differs from the paralysis of hypnotic-anaesthetic drugs with respect to the sequence of involvement of the corneal and pinna reflex; in anaesthesia, the former disappears first while muscle relaxants abolish preferentially the latter (Goodsell *et al.*, 1953). This is, however, not uniformly true (Witkin *et al.*, 1959).

All centrally acting muscle relaxant drugs with the exception of orphenadrine produce a reversible paralysis of voluntary muscles. This is not surprising since they were selected on the basis of efficacy in this test. It has, indeed, been the most common test used probably because of its obvious convenience. Its use sprang from the belief that a drug which possesses specific muscle relaxant properties will eventually, in larger doses, produce motor paralysis (Roszkowski, 1960). There is, however, no compelling neurophysiological evidence to support this thesis as there is no compelling evidence against it. The intimate mode of action of these drugs is not known nor have the physiological mechanisms upon which these drugs act been sufficiently elucidated. It is, therefore, only fair to regard motor paralysis as a possible but in no way rigid characteristic of drugs which produce a specific relaxant action in therapeutic doses. The example of orphenadrine is one case in point. The fact that the degree of paralysing action of a relaxant does not necessarily correspond to its clinical efficacy is another (Berger and Riley, 1949).

There are a number of other simple procedures used to reveal motor deficits short of paralysis, such as the inability of the mouse to cling to a

slowly rotating rod or an inclined plane, or its failure to grasp objects and hold on to a ledge. It is obvious, however, that drug-induced motor deficiencies are not intended to occur clinically. The same is certainly true for motor paralysis itself. It is from this perspective as well that the validity of such criteria is debatable. The use of the righting reflex test is, on the other hand, meaningful if neuromuscular blocking or anaesthetic activity is measured: motor paralysis is, in that case, the main or a portion of the main action of the drug tested. It is not suggested to dismiss the righting reflex test from the screening for central muscle relaxant activity but to recognize its limitations.

Mephenesin-like compounds produce a reversible motor paralysis of the ascending type not only in the mouse but also in other species such as the rabbit, cat and dog. In trying to advance an explanation for this paralysis, one of a few possibilities may be considered. It has been shown that mephenesin does block monosynaptic pathways in higher doses (Kaada, 1950; Taverner, 1952; Latimer, 1956). Stretch reflexes in antigravity muscles depend on monosynaptic spinal circuits; when the latter cease to function, an erect position can no longer be maintained. The doses of mephenesin which block monosynaptic pathways are within the range of those which abolish the righting reflex in the intact animal. Orphenadrine, on the other hand, does not block monosynaptic pathways: the knee jerk does not start to decline until the animal dies from vasomotor collapse following a lethal dose of the drug. Orphenadrine fails to produce motor paralysis in the mouse, rabbit, cat and dog (in toxic doses it precipitates clonic convulsions with intermittent periods of prostration). The effect on monosynaptic spinal reflexes might be the key to the difference between these two drugs with respect to motor paralysis.

Blockade of Polysynaptic Reflexes

The preferential blocking effect of mephenesin on polysynaptic spinal reflexes such as the flexor or crossed extension reflex whose reflex arcs include spinal interneurons, led del Castillo (1948) and Henneman *et al.* (1949) to consider 'the internuncial cell' as the specific site of action of mephenesin. As the number of mephenesin-type compounds exhibiting polysynaptic reflex blockade grew, designations like polysynaptic blocking agents or interneuronal depressants were adopted to signify what was believed to be the intimate mechanism by which these drugs exerted their spectrum of pharmacological and clinical activities.

This terminology with its far-reaching implications is unfortunate and has already provoked critical comment (Domino, 1964; Esplin, 1965). First of all, one has to realize that interneurons are plentiful at all levels of the central nervous system and that the majority of nervous processes are dependent on polysynaptic pathways. Consequently, most drugs which act on the central nervous system, do so by affecting, of necessity, certain

interneurons and polysynaptic circuits. Hence, many will be interneuronal blocking agents of some kind or another. The great variety of effects displayed by drugs acting upon central nervous structures may be viewed as a reflection of different sensitivities, quantitatively as well as qualitatively, among neurons, interneurons and pathways of different levels of organization. It is the specific affinity of drugs to certain neuronal elements and systems composed thereof, which will determine their action.

Mephenesin, for example, blocks the polysynaptic pathways of certain spinal and bulbar reflexes but it does not depress the multineuronal circuits of the ascending reticular formation responsible for arousal and wakefulness. Barbiturates depress both multineuronal systems, orphenadrine depresses neither. Carisoprodol, though chemically resembling mephenesin, behaves like orphenadrine (del Castillo and Nelson, 1960). In addition to the crossed extension reflex elicited by stimulation of the contralateral sciatic nerve, mephenesin and related agents depress the facilitation of the knee jerk which is elicited by contralateral sciatic stimulation also but at lower voltages (Abdulian *et al.*, 1960). Orphenadrine abolishes the facilitation of the knee jerk but does not affect the crossed extension reflex, both being polysynaptic. Both mephenesin, and orphenadrine, depress the polysynaptic pathways mediating reticular facilitation and decerebrate rigidity, but only mephenesin depresses the polysynaptic pathways mediating reticular, cerebellar, caudate and cortical inhibition. Carisoprodol acts again like orphenadrine in this respect (del Castillo and Nelson, 1960). Mephenesin, carisoprodol and other centrally acting muscle relaxants alleviate a variety of clinical states of skeletal muscle hypertonia and so does orphenadrine. A number of unrelated agents like morphine, trimethadone and others (De Salva and Oester, 1960; Goodman *et al.*, 1946), depress polysynaptic spinal reflexes but fail to exhibit muscle relaxant activity.

It is clear that the foregoing examples apply within certain dose ranges of the drugs discussed. The list could be extended in various directions. The examples given should, however, suffice to illustrate the points to be made: all drugs mentioned have central nervous effects and do have interneuronal (or polysynaptic) blocking activities in one system or another; their designation as interneuronal blocking agents would be redundant and misleading. Further, there does not exist a simple relationship between the blockade of polysynaptic reflexes and effectiveness against muscle spasm. Neither does there exist a convincing physiological reason that there should be one. The abolition of polysynaptic reflexes by a drug does neither indicate nor explain its value as a muscle relaxant. The use of the flexor or crossed extension reflex is of little predictive significance with respect to the therapeutic potentiality of a drug.

Anti-strychnine Activity

The efficacy of mephenesin and related compounds in antagonizing

strychnine convulsions and death has been interpreted in support of the concept of elective interneuronal blockade. However, the anti-strychnine activity did not parallel the depression of spinal interneuronal pathways by these agents (Berger, 1949). Anti-strychnine activity has further been thought to be characteristic of drugs possessing specific muscle relaxant properties. Orphenadrine, carisoprodol and phenyramidol which do have such properties, are devoid of an antagonistic effect against strychnine. Conversely, methysticin and analogues which were found to be potent antagonists of strychnine, failed to produce skeletal muscle relaxation in man (Klohs *et al.*, 1959). Various anticonvulsant drugs do also possess varying degrees of anti-strychnine activity but have no specific relaxant properties. On the other hand, relaxant drugs do have varying degrees of anticonvulsive activity against metrazol, electroshock, etc. Hence, one is led to conclude with Domino (1964) that anticonvulsant and mephenesin-like activity are not related in any simple fashion.

The antagonism of mephenesin against strychnine claimed to occur well below the toxic dose range of the drug (Berger, 1947) but confirmed only at $\frac{2}{3}$ LD_{50} of mephenesin (Davis *et al.*, 1952), prompted Berger (1949) to designate mephenesin and related agents as spinal cord depressants. This designation which, on the one hand, is too limited, since both strychnine and mephenesin have sites of action above the spinal cord as well, implies, on the other hand, a generalization that invites comments along the same lines as have been made earlier. In conclusion, it can be said that the ability of a compound to counteract strychnine convulsions is not a precise indicator of central muscle relaxant activity.

Characterization of Centrally Acting Skeletal Muscle Relaxants by the Blockade of Facilitatory Motor Systems

It has been shown in the preceding discussion that none of the three activities discussed, i.e. motor paralysis, polysynaptic reflex blockade and strychnine antagonism, is truly characteristic of central muscle relaxant properties. Furthermore, these actions lack quantitative correlations with therapeutic effects as well as with each other. Their frequent occurrence among muscle relaxants (Table 1 routine tests) may be due to the preferential use of the respective test procedures in the search for new compounds. The complete ineffectiveness of orphenadrine and the partial ineffectiveness of carisoprodol in these tests stress their limitations further.

More sophisticated experimentation involving motor facilitation through brain-stem stimulation or decerebration, imitating clinical spasticity, has been available (Niemer and Magoun, 1947; Lindsley *et al.* 1949) but was rarely used. Berger (1959) and Berger *et al.* (1960) advocated the use of decerebrate rigidity as a fourth screening procedure for centrally acting skeletal muscle relaxants but only a few compounds were evaluated in this way (Table 1). Studies of drug effects on facilitatory mechanisms have been

equally scanty (Henneman *et al.*, 1949; Kaada, 1950; del Castillo and Nelson, 1960). Orphenadrine is effective in relieving decerebrate rigidity and in blocking the facilitation of the patellar reflex elicited at spinal and supraspinal levels (Ginzel, 1965 and 1966). Orphenadrine has these pharmacological activities in common with other muscle relaxants such as mephenesin and carisoprodol. Since these actions appear to be more representative of the therapeutic effect on muscle spasm than motor paralysis or the blockade of spinal reflexes and strychnine convulsions, a comparative study of several muscle relaxants currently in use seemed to be indicated.

Some 20 experiments were performed on cats under chloralose anaesthesia (80 mg./kg. i. p.). Facilitatory points in the brain stem reticular formation were stimulated with bipolar electrodes for 40 sec. to produce increases in the knee jerk which constituted 70–90 per cent of the maximal response obtainable (Ginzel, 1966). Water soluble drugs were injected intravenously and intraperitoneally, insoluble agents only intraperitoneally. The doses (in mg./kg.) which just abolished the facilitatory increments of the knee jerk were determined. A comparison of relative potencies (orphenadrine=100 per cent) is presented in Table 1.

It can be seen that all drugs tested were effective in abolishing the increase in the patellar reflex elicited by reticular facilitation. The knee jerk itself remained unchanged. The relative pharmacological potencies parallel closely the relative reciprocal values of maximal clinical doses used as an approximation to clinical efficacy for which no direct data exist.

It may be concluded that the central muscle relaxant activity is satisfactorily characterized by the blockade of reticular facilitation. A variety of pharmacological agents such as atropine, scopolamine, mecamylamine, diphenylpyraline, amphetamine and morphine, which have no known relaxant action, failed to affect the facilitation of the knee jerk.

Increased extensor tonus following intercollicular decerebration is caused by an enhanced activity of the reticular facilitatory region similar to that obtained by electrical stimulation of this area. The use of decerebrate rigidity for the detection of central muscle relaxant properties is also adequate but the degree of facilitatory activity is less consistent and reproducible in decerebrate preparations than in intact cats in which measured facilitatory stimulations can be applied. The decerebrate cat has, on the other hand, the advantage of not requiring anaesthesia. The use of both experimental procedures would be desirable. It is recommended that the activity observed in the intact animal under anaesthesia be confirmed in a non-anaesthetized preparation.

The depression by mephenesin of the facilitation of the patellar reflex without concomitant depression of the reflex itself has been interpreted as a selective blocking effect on interneurons (Henneman *et al.*, 1949 and Funderburk *et al.*, 1953). The depression of polysynaptic spinal reflexes by mephenesin has led to the same conclusion. It followed that both phene-

TABLE 2

	Blockade of		
	Polysynaptic spinal reflexes	Reticular facilitation	Clinical muscle relaxant efficacy
Mephenesin	+	+	+
Orphenadrine	—	+	
Morphine	+	—	—

mena should be affected by an 'interneuronal blocking agent' at the same time. This, however, is not the case: drugs which affect the pathways of polysynaptic reflexes do not necessarily affect the pathways of reticulospinal or spinal facilitation. An illustration of such behaviour is given in Table 2.

This example also points to the correspondence between depression of facilitation and clinical efficacy; such correspondence is lacking with respect to polysynaptic reflex blockade. The differential action of drugs on these two pathways is in no way inconceivable. A glance at the diagram by Lloyd (1941) depicting a spinal cross-section, with its intricate neuronal organization, convinces one that there does exist a number of segmental interneurons sufficient to serve different pathways. Electrophysiological studies (Eccles *et al.*, 1954; Frank and Fuortes, 1956 and Kolmodin, 1957) support the possibility that these interneurons are diverse enough as to exhibit differential sensitivities to drugs. This is further strengthened by the work of Longo (1961) who recorded from single spinal interneurons and found that mephenesin depressed the electrical activity in only 60 per cent of the recordings.

CONCLUDING REMARKS

The foregoing discussion on techniques for the identification of central muscle relaxant properties is in no way complete. An exhaustive treatment of the subject was aptly undertaken only recently (Domino, 1964). The intention here has been to concentrate on a few prominent issues and to introduce some fresh points of view.

A delineation of neuropharmacological correspondences with the clinical effectiveness of drugs in alleviating muscle spasm has been attempted. It appears that the depression of reticular facilitation of motor function by a drug holds a reasonable promise to indicate its potential clinical activity as a muscle relaxant. It is, however, abundantly clear that the clinical conditions and causes of 'muscle spasm' are varied and cannot be fully matched by any single mechanism study or drug screening procedure.

The intimate mechanism underlying the pharmacological activity of muscle relaxants is not known. An involvement of interneurons was expected

and was indeed demonstrated. However, little is gained for the understanding of pharmacological drug action unless these interneurons are identified morphologically and functionally, and possibly also with respect to their physical and/or chemical interactions with the drug. Further, it will be desirable to elucidate the role of the gamma loop in drug-induced muscle relaxation (Granit, 1964). Information in these areas will unquestionably bring forth the diversities inherent in the various compounds which are presently, and will until then be, listed and discussed under the one heading of centrally acting skeletal muscle relaxants.

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REFERENCES

1. Abdulian, D. H., Martin, W. R. & Unna, K. R., *Arch. int. Pharmacodyn.*, **128** (1960), 169.
2. Berger, F. M., *Br. J. Pharmac.*, **2** (1947), 241.
3. Berger, F. M., *Pharmac. Rev.*, **1** (1949), 243.
4. Berger, F. M., The Pharmacology and Clinical Usefulness of Carisoprodol, Wayne State Univ. Press, (1959).
5. Berger, F. M. & Bradley, W., *Br. J. Pharmac.*, **1** (1946), 265.
6. Berger, F. M., Kletzkin, M., Ludwin, B. J., Margolin, S. & Powell, L. S., *J. Pharmac. exp. Ther.*, **127** (1960), 66.
7. Berger, F. M. & Riley, R. F., *J. Pharmac. exp. Ther.*, **96** (1949), 269.
8. Buller, A. J., Symposium on Skeletal Muscle Spasm, Franklyn Ward and Wheeler, 1961, p. 1.
9. Cronheim, G., *Fortschr. Med.*, **82** (1964), 697.
10. Davis, H., Hine, C. H., Neal, M. W., Christensen, H. E. & Murphy, F. J., *Arch. int. Pharmacodyn.*, **89** (1952), 145.
11. del Castillo, J., *Trab. Inst. nac. Cienc. med. Madr.*, **12** (1948), 363.
12. del Castillo, J. & Nelson, R. E. (Jr), *Ann. N. Y. Acad. Sci.*, **86** (1960), 108.
13. de Maar, E. W. J., *Arch. int. Pharmacodyn.*, **105** (1956), 349.
14. de Salva, S. J. & Oester, Y. T., *Arch. int. Pharmacodyn.*, **124** (1960), 255.
15. Domino, E. F., Evaluation of Drug Activities, *Pharmacometrics*, **1** (1964), 313.
16. Eccles, J. C., Fatt, P. & Koketsu, K., *J. Physiol.*, **126** (1954), 524.
17. Esplin, D. W., The Pharmacological Basis of Therapeutics, 1965, ed. 3, Chap. 14, p. 237.
18. Frank, K. & Fuortes, M. G. F., *J. Physiol.*, **131** (1956), 424.
19. Lundeberg, W. H., King, E. E., Domino, E. F. & Unna, K. R., *J. Pharmacol. exp. Ther.*, **107** (1953), 356.
20. Ginzel, K. H. *Fed. Proc.*, **24** (1965), 517.
21. Ginzel, K. H., *J. Pharmac. exp. Ther.*, (1966) (in press).
22. Goodman, L. S., Swinyard, E. A. & Toman, J. E. P., *Fed. Proc.*, **5** (1946), 179.
23. Goodsell, J. S., Toman, J. E. P., Everett, G. M. & Richards, R. K., *J. Pharmacol. exp. Ther.*, **110** (1953), 251.
24. Granit, R., *Clin. Pharmac. Ther.*, **5** (1964), 837.
25. Hennerman, E., Kaplan, S. & Unna, K. R., *J. Pharmacol. exp. Ther.*, **97** (1949), 341.

26. Henneman, E. & Scherrer, J., *J. Pharmac. exp. Ther.*, **97** (1949), 342.
27. Kaada, B. R., *J. Neurophysiol.*, **13** (1950), 89.
28. Katz, F., *Proc. R. Soc.*, **155** (1962), 455.
29. King, E. E., Minz, B. & Unna, K. R., *Am. J. Physiol.*, **171** (1952), 738.
30. King, E. E., Minz, B. & Unna, K. R., *J. Comp. Neurol.*, **102** (1955), 565.
31. King, E. E. & Unna, K. R., *J. Pharmac. exp. Ther.*, **111** (1954), 293.
32. Kuohs, M. W., Keller, F. E., Williams, R. E., Toekes, M. I. & Cronheim, G. E., *J. med. pharm. Chem.*, **1** (1959), 95.
33. Kolmodin, G. M., *Acta Physiol. Scand., Suppl.*, **139** (1957), 40.
34. Latimer, C. N., *J. Pharmac. exp. Ther.*, **118** (1956), 309.
35. Lindsley, D. E., Schreiner, L. H. & Magoun, H. W., *J. Neurophysiol.*, **12** (1949), 197.
36. Literature Review: Norflex(R) Orphenadrine Citrate, *Curr. ther. Res.*, **6** (1964), 549.
37. Lloyd, D. P. C., *J. Neurophysiol.*, **4** (1941), 525.
38. Longo, V. G., *Arch. int. Pharmacodyn*, **132** (1961), 222.
39. Moruzzi, G. & Magoun, H. W., *Electroenceph. Clin. Neurophysiol.*, **1** (1949), 455.
40. Niemer, W. T. & Magoun, H. W., *J. comp. Neurol.*, **87** (1947), 367.
41. O'Dell, T. B., Wilson, L. R., Napoli, M. D., White, H. D. & Mirsky, J. K., *J. Pharmac. exp. Ther.*, **128** (1960), 65.
42. Roszkowski, A. P., *J. Pharmac. exp. Ther.*, **129** (1960), 75.
43. Schwab, R. S., *Practitioner*, **192** (1964), 104.
44. Taverner, D., *Br. J. Pharmac.*, **7** (1952), 655.
45. Witkin, L. B., Spitaletta, P. & Plummer, A. J., *J. Pharmac. exp. Ther.*, **126** (1959), 330.

5-Hydroxytryptamine and Antiepileptic Drugs

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Corne, Pickering and Warner (1963) reported that on intraperitone injection of 5-hydroxytryptophan (5-HTP), mice exhibit convulsive he twitches within 20–30 min. A similar convulsive head twitch can be produc by stimulation of the external auditory meatus with a mounted horse ha This reflex movement is multineuronal in character and has been designat as the pinnal reflex.

It has been thought that 5-hydroxytryptamine (5-HT) is incapable readily penetrating the blood-brain barrier (Hadgraft, Price and W 1958; Bhargava and Tangri, 1959), but 5-HTP may readily do so. 5-H is converted in the brain by enzymatic decarboxylation to 5-HT and then stored (Udenfriend, 1958; West, 1958). The convulsive movem has been ascribed to increased amounts of 5-HT in the brain.

It has been suggested that this phenomenon may be used for studie central actions of 5-HT and related drug actions.

The injections of diphenylhydantoin (Phenytoin) in small laborat animals produce a rise in the 5-HT content of the brain Anderson, Markov and Bonnycastle, 1962) and in view of the repetitive convulsive nature the head twitches, it was of interest to study the inter-relationship of a convulsant drugs with the phenomenon. The action of atropine was studied as it has been suggested that atropine may possibly modify behavi changes induced by injections of 5-HTP (Wada, Hill and Wrinch, 1963).

The following studies were conducted on mice. In prelimin experiments, the pinnal reflexes were elicited from both ears in group 6 mice, following which the animals were kept under observation for 3 min. for occurrence of spontaneous head twitches. Subsequently *dl*-5-H in saline was injected intraperitoneally in a dose of 250 mg. per kg. b weight. The total number of head twitches were counted continuously 21–25 min. The injection of 5-HTP produced sedation in the mouse but

did not prevent the appearance of head twitches. The pinnal reflexes were re-checked and found to be present at the termination of the experiment.

Groups of animals tested for pinnal reflex were pretreated after the usual period of observation for spontaneous head twitches with one of the following: (1) phenytoin sodium 25 mg./kg.; (2) phenytoin sodium 100 mg./kg.; (3) troxidone 325 mg./kg.; (4) phenobarbitone sodium 25 mg./kg.; (5) primidone 25 mg./kg.; (6) atropine sulphate 2·5 mg./kg.; and (7) saline in equivalent volume.

The animals were observed for 30 min.; during the last three minutes of which they were individually observed for presence of head twitches. The usual injection of 5-HTP was then administered intraperitoneally and the experiment was continued as described before.

The injections of phenytoin, phenobarbitone or mysoline produced minor to moderate sedation. The sedation usually became more marked after injection of 5-HTP. Troxidone produced marked sedation and this became profound after injection of 5-HTP. Some of the animals of this group lost their righting reflex. This state continued for 24–48 hr, at the end of which the animals of this group were dead.

The results are graphically illustrated in Fig. 1.

The average number of twitches was reduced after low doses of phenytoin, troxidone, atropine and phenobarbitone, but was increased after high doses of phenytoin and mysoline. The reduction after phenobarbitone was not significant. Thus it was seen that some of the anticonvulsant drugs reduced the number of head twitches, whereas others aggravated it. The same drug in higher doses increased the number of twitches, whereas lower dosages produced opposite effect.

Troxidone which had produced profound depression also abolished the multineuronal pinnal reflex. The low doses of phenytoin which had blocked the action did not affect the pinnal reflex and as such was relatively more specific.

Theoretically the drug induced modifications of the central actions of 5-HT, as seen after intraperitoneal injections of 5-HTP which could be due to an effect on (a) passage of 5-HTP through the blood-brain barrier, (b) enzymatic decarboxylation of 5-HTP to 5-HT, and (c) facilitation or antagonization of the effects of 5-HT.

The 5-HT content of the brain was extracted and assayed (Parratt and West, 1957) after intraperitoneal injections of 5-HTP and compared with similar values obtained in saline treated animals. The extraction was done with 95 per cent acetone to prevent simultaneous extraction of substance P. It was seen that there was a sharp rise in 5-HT content of the brain after intraperitoneal injection of 5-HTP (Fig. 2).

A similar rise was seen after treatment with phenytoin sodium (low dose), mysoline or phenobarbitone (Fig. 2). Injection of phenytoin, as such, in the low dose did not significantly alter the 5-HT content of the brain.

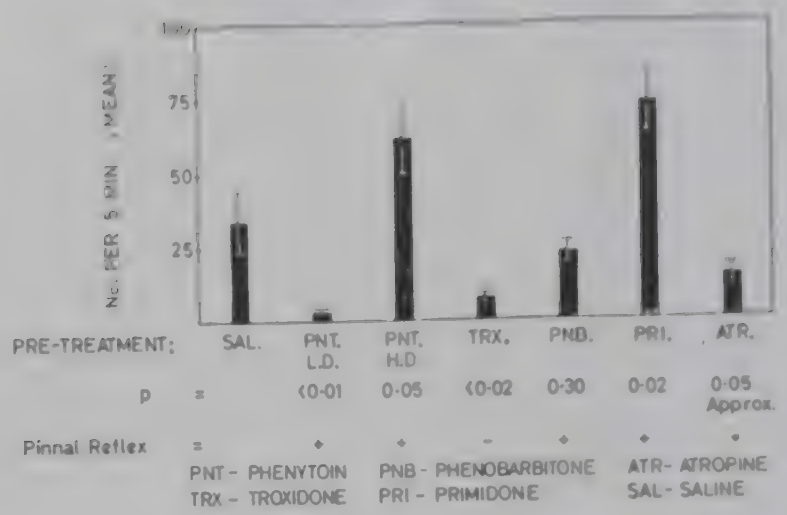
No. PER 5 MIN. (MEAN)
75
50
25
PRE-TREATMENT: SAL. PNT. L.D. PNT. H.D TRX. PNB. PRI. ATR.
P = <0·01 0·05 <0·02 0·30 0·02 0·05 Approx.
Pinnal Reflex = + + - + + +
PNT - PHENYTOIN
TRX - TROXIDONE
PNB - PHENOBARBITONE
PRI - PRIMIDONE
ATR - ATROPINE
SAL - SALINE

FIG. 1 — EFFECT OF VARIOUS DRUGS ON 5-HTP-INDUCED HEAD TWITCHES IN THE MOUSE

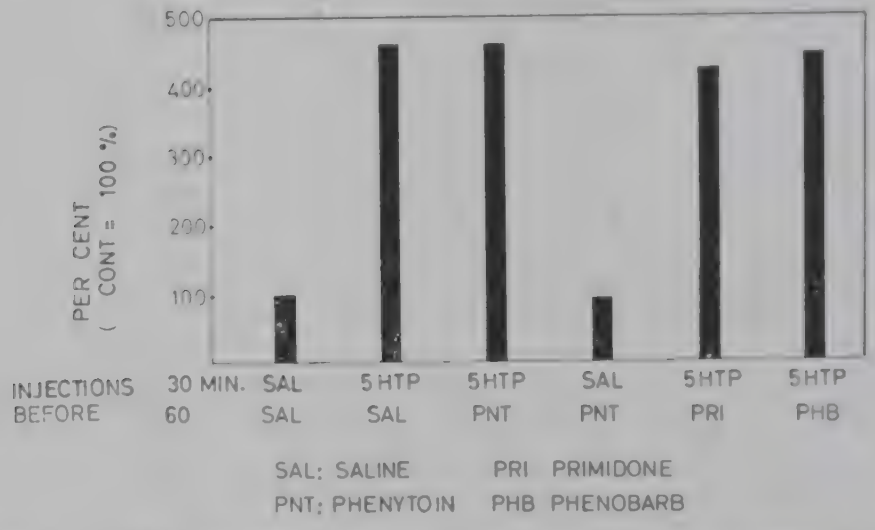

FIG. 2 — 5-HTP CONTENTS OF MOUSE BRAIN AFTER INTRAPERITONEAL 5-HTP AS COMPARED WITH CONTROLS AND THE EFFECT OF VARIOUS PRETREATMENTS ON THE SAME

Thus it was seen that these drugs did not influence the entry of 5-HTP in the brain and also did not affect the processes of enzymatic decarboxylation.

Phenytoin sodium in a concentration of 10 μg. per ml. considerably reduced the response of rat uterus or guinea-pig ileum to either 5-HT (Fig. 3) or acetylcholine (Fig. 4), but was without any effect on the response of guinea-pig ileum to histamine. The blocking action was reversible and there was immediate recovery of response when phenytoin was washed out. Phenytoin in 10 times higher concentration nearly blocked the response to both acetylcholine and 5-HT.

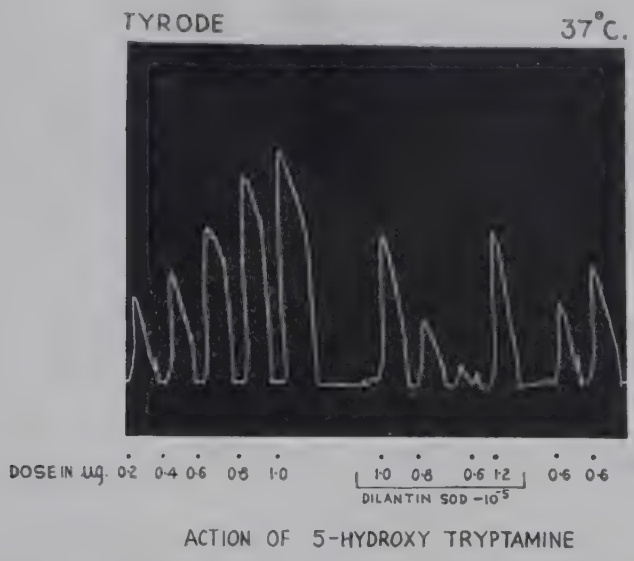

FIG. 3 — ACTION OF 5-HTP ON GUINEA-PIG ILEUM IN THE ABSENCE AND PRESENCE OF PHENYTOIN SODIUM

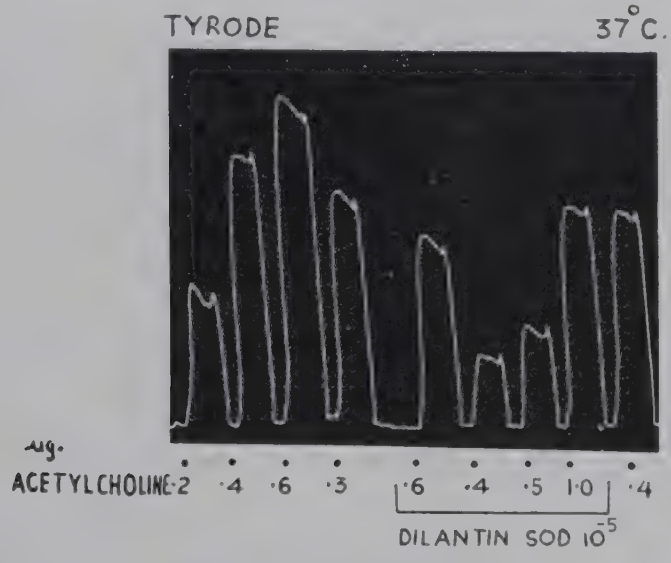

FIG. 4 — ACTION OF ACETYLCHOLINE ON GUINEA-PIG ILEUM IN THE ABSENCE AND PRESENCE OF PHENYTOIN SODIUM

Troxidone, phenobarbitone or primidone in equivalent concentrations, did not affect the response of the above tissues to either histamine, 5-HT or acetylcholine.

Corne, Pickering and Warner (1963) found that phenytoin potentiated the head twitch response. These authors injected the drug at a dose level of 69–102 mg. per kg., and the method of assessment was quantal, animals being observed at 24 and 25 min. for presence of at least one head twitch. The results have been confirmed by using the same dose, but applying a different way of measurement.

The surprising thing is that when about 25 per cent of the above dose of phenytoin was employed, there was actually a significant lowering in the count of head twitches. The inter-relationship of brain 5-HT and anticonvulsant drugs is rather complex. General electrical stimulation to produce convulsive seizures raises 5-HT content of the brain and this is associated with raised electroshock threshold (Brenner, Picchioni, Chin and Burton, 1961; Sofer and Gubler, 1962).

The effect of phenytoin on 5-HT level of the brain is controversial. Anderson, Markowitz and Bonnycastle (1962) have noted a significant rise after a dose of 12·5 mg./kg., whereas others have reported absence of any such action (Prockop, Shore and Brodie, 1959; Plan, Funderburk and Finger, 1961).

The effect of low doses of phenytoin in preventing central effects of 5-HT may be related to its anti-5-HT and anti-acetylcholine action. It has been reported that atropine modifies the behavioral changes induced by injections of 5-HTP in cats and monkeys (Wada, Hill and Wrinch, 1963), and a protection has also now been seen in mice. The potentiation of the central effects of 5-HT by higher doses of phenytoin or primidone is difficult to explain. The brain level of 5-HT after intraperitoneal 5-HTP was of the same order in primidone treated rats as in control animals, and this or other anticonvulsant drugs had no effect on peripheral actions of 5-HT. The different anticonvulsant drugs affected the central action of 5-HT in different manner; even different doses of the same drug produced opposite results on central actions of 5-HT. It would thus appear that the mechanism of anticonvulsant actions is not likely to be related as closely to an effect on the central actions of 5-HT as was previously supposed.

REFERENCES

1. Anderson, E. G., Markowitz, S. D. & Bonnycastle, D. D., *J. Pharmac.*, **136** (1962), 179.
2. Bhargava, K. P. & Tangri, K. K., *Br. J. Pharmac.*, **14** (1959), 411.
3. Brettner, C., Picchioni, A., Chin, L. & Burton, L. L., *Diseases of the Nervous System* **22** (1961), 93.
4. Corne, S. J., Pickering, R. W. & Warner, B. T., *Br. J. Pharmac.*, **20** (1963), 106.
5. Hadgraft, J. W., Price, S. A. P. & West, G. B., *J. Pharmac.*, **10** suppl. (1958), 87T.
6. Parratt, J. R. & West, G. B., *J. Physiol.* (Lond.), **137** (1957), 169.
7. Plan, S. Y., Funderburk, W. H. & Finger, K. F., *Fed. Proc.*, **20** (1961), 680.
8. Sofer, S. & Gubler, C. J., *Fed. Proc.*, **21**(1), (1962), 340.
9. Udenfriend, S., 5-Hydroxytryptamine (ed. G. P. Lewis) Pergamon Press, London (1958).
10. Prockop, D. J., Shore, P. A. & Brodie, B. B., *Ann. N. Y. Acad. Sci.*, **80** (1959), 643.
11. Wada, J. A., Hill, D. & Wrinch, J., *J. Neuropsychiatry*, **4** (1963), 413.
12. West, G. B., *J. Pharmac.*, **10** suppl. (1958), 92T.

Chemistry and Pharmacology of Azetidinones

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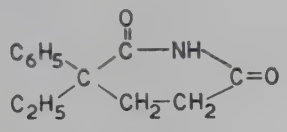
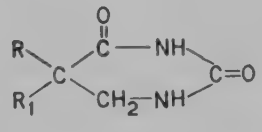
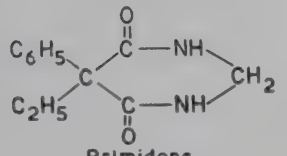
Azetidin-2-ones and β-lactones form a particularly significant class of compounds in the framework of a systematic search aimed at identifying substances active on the central nervous system. In fact, from the point of view of studying the relationship between activity and structure, they can be formally related, through expansion of the ring, to glutethimide[1] (Doriden$^{(R)}$, hypnotic—Ciba), to dihydrouracils[2] active as hypnotics to primidone widely used as an anti-epileptic drug[3,4] (Mysoline$^{(R)}$, ICI), and to other compounds.



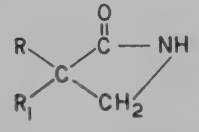
Glutethimide



Dihydrouracils

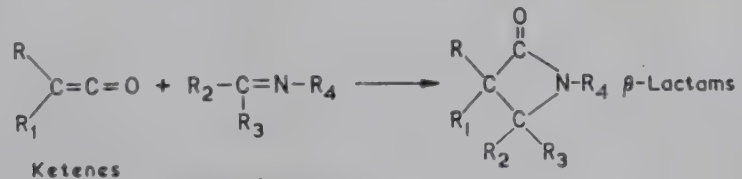


Primidone



β-Lactams (azetidin-2-ones)

The first authentic azetidin-2-one was obtained by Staudinger[5] in 1907; a dozen members of the same class were prepared within the next few years



Ketenes

Schiff's bases

by this investigator and his colleagues. The method used – reaction of ketones with Schiff's bases – permitted only the synthesis of derivatives necessarily substituted at the nitrogen.

These compounds were, for very many years, considered as laboratory curiosities; no further research, with the exception of a neglected work by Breckpot[6] in 1923, was published on this subject until after the Second World War. Considering, in particular, a presumed but not demonstrated instability of four-atom heterocyclic compounds, no research was undertaken as to possible industrial uses and biological activities of azetidin-2-ones. Only after unravelling penicillin's structure, which presents a β-lactam structure in the molecule, did interest centre on these compounds and work on them gradually intensified. On the one hand, Sheehan and his team started a massive synthesis attack which, also through study of the synthetic possibilities in the β-lactam field, culminated in total synthesis of a penicillin, described in 1959[7]. In 1956, my team started research on this subject with the aim of studying the neglected aspects of azetidin-2-one chemistry, with the further intent of revealing the potential activity of these compounds on the central nervous system. Obviously, a subsequent study of the relationship between the activity, if this came to light, and the chemical structure, was the logical sequence. Moreover, important contributions to β-lactam chemistry have been made during the last few years by some East European investigators, especially by the Russians, Knunyants and Gambaryn, and by the Bulgarian, Spasov. The published results, which we shall deal with later, appear to show that these research workers are also studying β-lactams as compounds potentially active on the central nervous system. Very recently, all the information available on azetidin-2-ones published up to 1962 has been excellently collected by Moore[8] in his review and reference should be made to this for all further details.

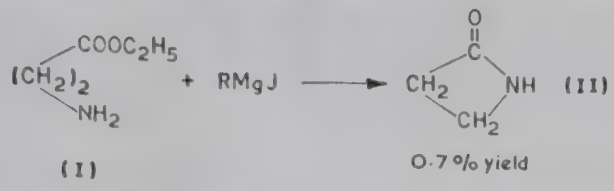
We would, however, like to mention the most significant aspects and the results of our chemical and pharmacological studies in this field. The study undertaken in 1956 is still in progress. Nevertheless, a review of the research already completed can be made and some general conclusions can be reached.

Chemistry of Azetidin-2-ones

When the present research work was started, only two azetidin-2-ones non-substituted at the nitrogen were known. The first is the parent compound of the class and was obtained, with a very poor yield, by Holley and Holley[9] by allowing ethyl β-aminopropionate (I) to react with Grignard reagent. In actual fact, this is an application of the method described by Breckpot[6,*] in 1923 and it is very probable that this technique was previously unknown to the American authors.

*Cyclisation of β-aminoesters to β-lactams forms a special case of amide synthesis according to Bodroux [*C. R. hebd Seances Sci.*, **138** (1904), 1427].

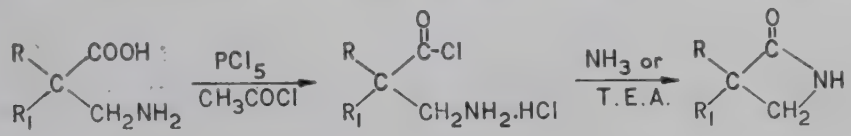
However, this subject will be dealt with further in the present report.



The second compound is 3-(*N*-α-pyridone)-azetidin-2-one hydrobromide obtained by Adams and Jones[10], with excellent yields, by the action of hydrobromic acid on α′-(*N*-α-pyridone)-β-aminopropionic acid in ethanol solution. This cyclization technique is not suitable for general use.

It was considered indispensable to have azetidin-2-ones unsubstituted at the nitrogen available for our programme. As a result, the first thing to be done was to undertake a synthetic study in order to obtain the required compounds.

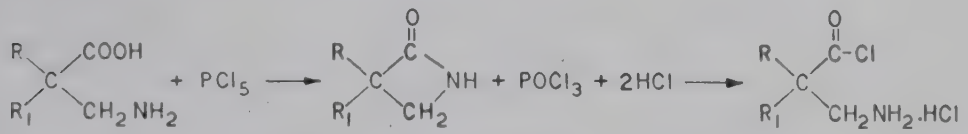
The first synthesis carried out by our group[11] consisted in making ammonia or triethylamine to react with α, α-disubstituted β-aminopropionic acid chloride hydrochloride, which was obtained using Emil Fischer's method[12] for the preparation of α-amino acid chloride hydrochlorides.



$R = C_6H_5$ $R_1 = C_2H_5, CH_3, n.C_4H_9, i.C_4H_9, CH_2C_6H_5, C_6H_5$, cyclohexyl

$R = C_2H_5$ $R_1 = C_2H_5, CH_2C_6H_5$, cyclohexyl

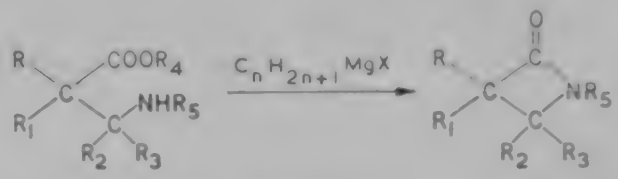
We believe it highly probable that azetidin-2-ones are formed as intermediates, during the reaction of β-aminopropionic acids with PCl_5. The hydrochloric acid which forms, reopens the β-lactams (demonstrated reaction) giving rise to the hydrochloride of the acid chlorides which are isolated. This reaction mechanism has been demonstrated in the case of 3, 3-diphenyl derivatives.



A similar procedure was developed by Blicke and Gould[13] practically at the same time. The yields obtainable with this technique are satisfactory only in the case of the 3, 3-disubstituted derivatives, while they do not exceed 4 per cent for the 3-phenyl mono-derivative[14]. This procedure, although of limited application, has been found to be extremely useful in subsequent experiments to synthesize azetidin-2-ones not obtainable in any other manner[15] [for instance: 3-(*p*-nitrophenyl)-3-ethylazetidin-2-ones].

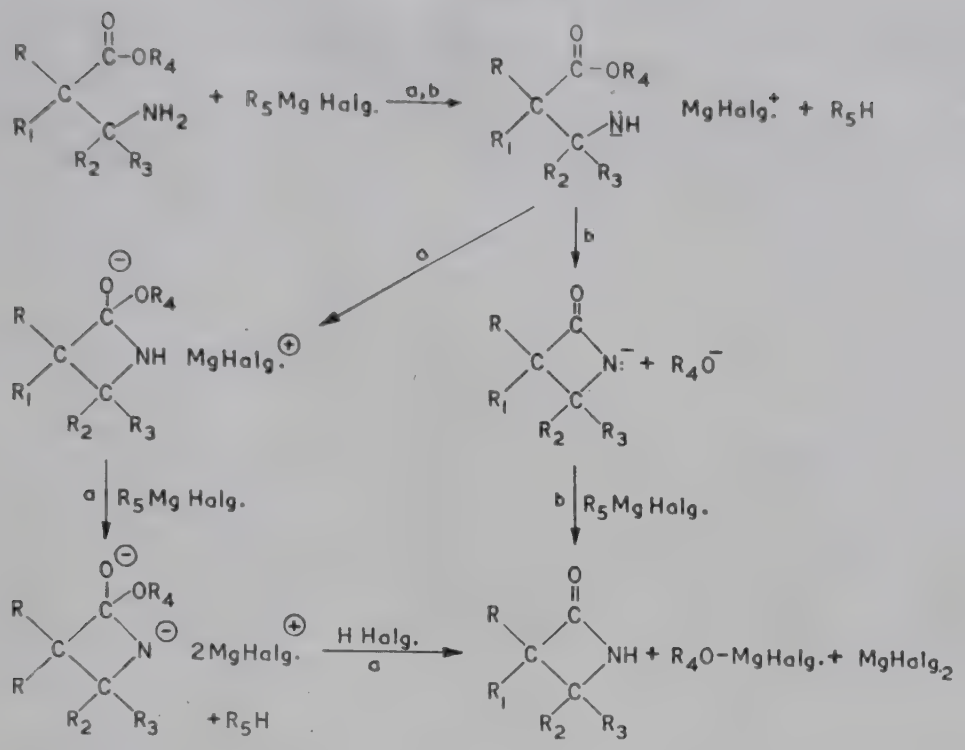
The compounds thus obtained were submitted to pharmacological screening and the working hypothesis was found to be valid. 3-amino β-lactams have displayed a depressant action on the central nervous system, in particular, 3-phenyl-3-ethylazetidin-2-one has been found to be active as a classical type of hypnotic[16–18]. It was therefore decided to extend the chemical research and consequently, it was necessary to study other synthetic procedures.

A second, much widely applied cyclization procedure developed by us consists of allowing the esters of β-aminopropionic acids, variably substituted at α or β, to react with alkylmagnesiumhalogenides (Grignard reagent) in ether, tetrahydrofurane or ether-benzol mixture[19–22].



By systematic study of the parameters[11,19,23], we have determined the optimal condition ensuring the best yields of β-lactams mono or disubstituted at C_3 or at C_4, both in the N-alkyl and N-unsubstituted series. In general terms, it has been observed that the reaction develops optimally with 2 moles of reagent (3 in the case of 3-mono-aryl derivatives), at low temperature (0–5° C.) when aromatic substituents are present, and at 80° C. if aliphatic substituents on carbon atoms 3 and 4 only are present. The cyclization yields are all the higher, the heavier the substitution. Usually, larger quantities of disubstituted compounds, compared with monosubstituted derivatives are obtained and the presence of mono-aromatic substituents permits better yields than mono-aliphatic ones. Cyclization at high temperature transforms the compounds with aromatic groups at C_3 into polyamides soluble in benzol. This polymerization phenomenon takes place each time the 3-phenyl-azetidin-2-ones are treated in solution under heating with reagents such as sodamide sodium hydride, sodium alcoholate, etc. Graf *et al.* of Farbwerke Hoechst, have developed an industrial process for the synthesis of polyamides starting from 3, 3-dimethylazetidin-2-ones[24] which are of potential interest to the textile field.

The reaction mechanism which, bearing in mind the experimental requirements, in our opinion best explains cyclization of β-aminopropionic acid esters into β-lactams[19] is indicated in the following scheme a). Moore[8] considers that "the postulation of this (the dianion) presumably high-energy species is unnecessary, since the azetidinone initially formed by displacement of the amide ion contains a more acidic hydrogen atom than the starting amine and would immediately consume a second molar equivalent of Grignard reagent". According to this investigation, the reaction mechanism is that indicated as (b).



It has been possible to synthesize a large number of new azetidin-2-ones by means of this process, in particular 3 and 4 mono-alkyl and mono-aryl substituted β-lactams[14,21,25,26] and 3, 3- and 4, 4-di-alkyl and aryl-alkyl-ones[19,20,22].

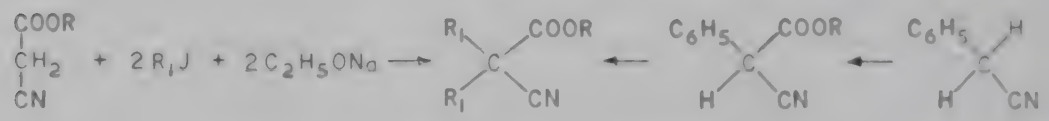
It has also been possible to obtain 3-phenyl-3-hydroxy-methyl-azetidin-2-one[27] and 3-phenyl-3-hydroxyazetidin-2-ones[28]. The synthesis has, moreover, been found suitable for obtaining *N*-alkyl derivatives of this class[29]. In this case, the most complicated aspect of the problem is the synthesis of *N*-monosubstituted β-aminopropionic esters. During the present research, two enantiomorphs of the hypnotic 3-phenyl-3-ethyl-azetidin-2-one[30] were prepared; this is the first example of an optically active β-lactam. The pharmacological activity of the two isomers is practically equal. Finally, it was necessary to perfect two β-lactam colorimetric reactions[31]. These are based on colour reactions of the β-amino acids formed by mild hydrolysis, with ninhydrin, or on reaction of azetidinones with sodium nitroprusside. Both can be used for the detection and determination of azetidinones in biological fluids.

Obviously, the azetidin-2-ones prepared according to this second process were also submitted to pharmacological screening. No improvement of the 3-phenyl-3-ethyl derivatives in hypnotic action over that could be revealed. Nevertheless, a new activity, which we shall refer to later, appeared; 1-methyl-3-phenyl-3-ethylazetidin-2-one (L 1743) possesses marked myorelaxant activity, qualitatively and quantitatively comparable with that of meprobamate.

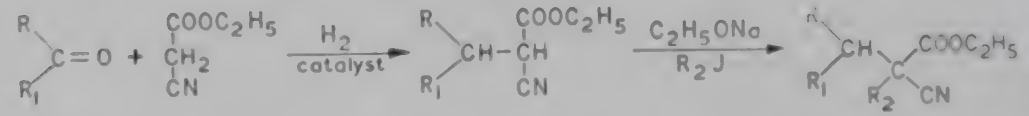
From the chemical point of view, it is interesting to observe that the

key products for the synthesis of azetidin-2-ones, according to the two processes here described, are the β-aminopropionic acids, variously substituted in position α and β, and the corresponding esters. These compounds are obtained by catalytic reduction of the corresponding cyanoacetates, variously substituted, which also form the starting point for the third synthesis we have perfected (*see* below) as well as for the preparation of azetidine-2, 4-diones with hypnotic action[27,28]. This last class of compounds will not, however, be considered here; this is quite another aspect of our studies on the CNS. We, therefore, wish to describe the techniques used for the synthesis of 3-aminopropionic acids giving the fullest details.

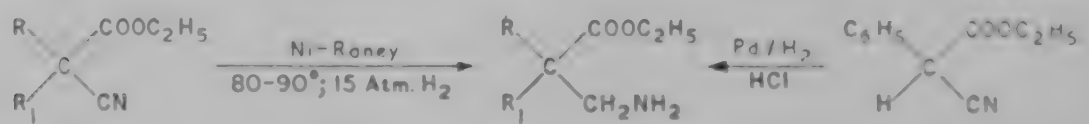
Ethyl cyanoacetate may be easily di-alkylated by means of alkyl halogenides and sodium ethylate, giving ethyl α, α-di-alkyl-β-cyanoacetates. Benzyl cyanide comes, instead, in the first place, carbethoxylated with ethyl carbonate and subsequently alkylated as stated above.



Ethyl cyanoacetate is also the starting point for the synthesis of α, α-nonsymmetrical substituted cyanoacetate derivatives. It is allowed to react with an aldehyde or ketone in an atmosphere of hydrogen, in the presence of suitable basic catalysts so as to saturate gradually the double bond which forms. The product is then alkylated in the usual way.

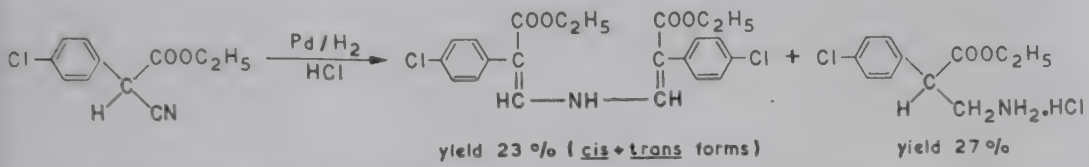


Catalytic reduction of the substituted cyanoacetate thus obtained presents many problems. Two cases essentially occur: i) if two substituents are present at C_2, whether aromatic or aliphatic is not important, or a single aliphatic substituent, reduction is carried out at 80–90° C. with Raney nickel catalyst and hydrogen at a pressure of 15–20 atmospheres[19,26–28]; ii) if, instead, a single aromatic substituent is present at C_2 the reduction must be performed with Pd on charcoal and hydrogen at ordinary pressure in a hydroalcoholic medium containing hydrochloric acid[14,26].



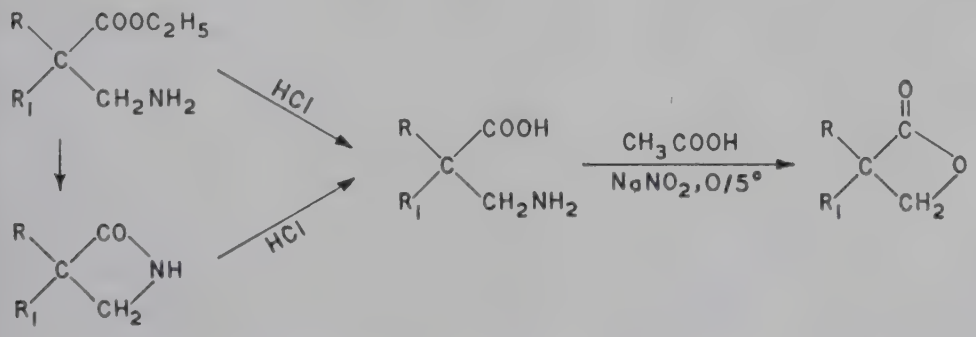
Reduction in the presence of Pd also leads to interesting secondary products of *bis*-enaminic structure and in different stereoisomeric forms. In the case of the 3-phenyl derivative, only low yields (2-2–3) of these compounds are formed while 3-phenyl derivative with halogen atoms

Cl, F, etc.) substituted on the aromatic nucleus, are produced to a much greater extent. A plausible explanatory mechanism for this reaction has been given by us in detail[34].

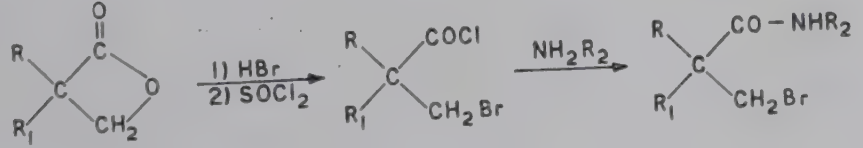


Ethyl β-aminopropionates variously substituted in α may be hydrolysed in acid medium directly[35,36,11] or after transformation into β-lactams[37]. This gives the β-aminopropionic acids which form the starting point for the third synthetic process to azetidin-2-ones developed in our laboratories. The amino acids are thus diazotized in 25 per cent acetic acid at 0/ +5° C. and the 3-substituted β-propiolactones[38] are obtained. The yield is generally good and in any case is all the larger, the heavier the substitution at C_3[35–37]. This is a class of compounds only slightly studied precisely because of the lack of suitable methods of synthesis; however, they are of enormous potential interest for the preparation and study of whole new classes of compounds.

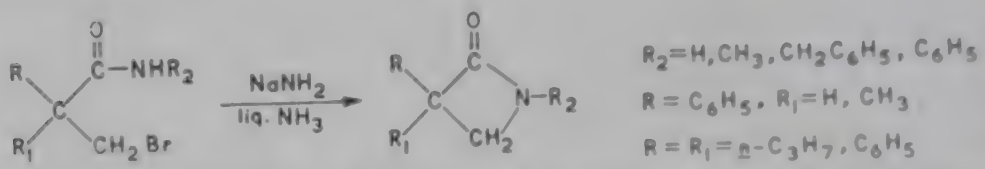
An alternative process for the preparation of α, α-disubstituted β-lactones consists in cyclization of α-substituted β-hydroxypropionic acids, obtainable by different processes[37,41] using cyclohexylcarbodiimide[39] or thionylchloride[40]. The β-propiolactones are either low melting point solids or liquids and show a characteristic band at 1810–1820 cm.$^{-1}$ on I.R. spectrography. They have an irritating action on the skin and mucosae and must be handled with care.



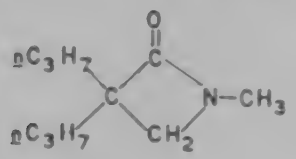
Beta-propiolactones hydrolyse with dry hydrobromic acid giving rise to the formation of β-bromopropionic acids, with good yields, which are easily transformed into the corresponding acid chlorides[12]. The latter obviously react with ammonia or aromatic amines, thus giving the corresponding β-halogenated propionamides.



In previous works, Knunyants and Gambaryan[45-47] obtained the corresponding N-substituted azetidin-2-ones by cyclization of seven N-substituted β-bromo-β-phenylpropionamides. The same investigators subsequently[45] described the cyclization of α,α-diphenyl-β-bromopropionamide using sodium ethylate, sodium isopropylate, caustic soda and potash, obtaining 3,3-diphenylazetidin-2-one. In analogy to this work, we treated our β-bromopropionamides with one mole of sodamide in liquid ammonia, obtaining excellent yields of N-substituted and N-unsubstituted azetidin-2-ones[46].



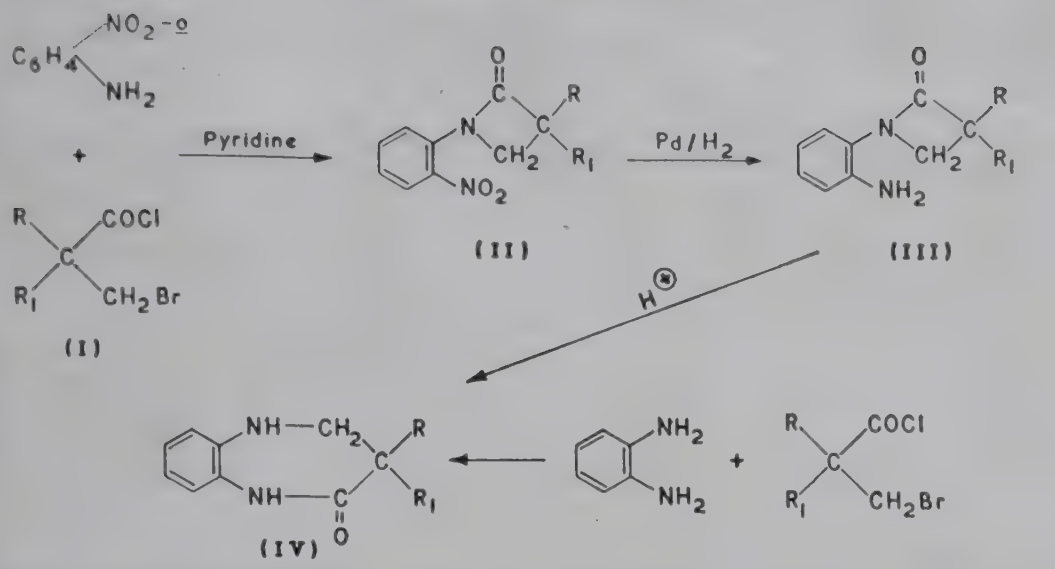
During this research we obtained a particularly interesting compound for our survey of substances active on the central nervous system, 1-methyl-3,3-di-*n*-propylazetidin-2-one (L 3432), which acts, when administered orally and parenterally, on the behaviour of animals, although differing from all other known tranquillizers from the pharmacological point of view.



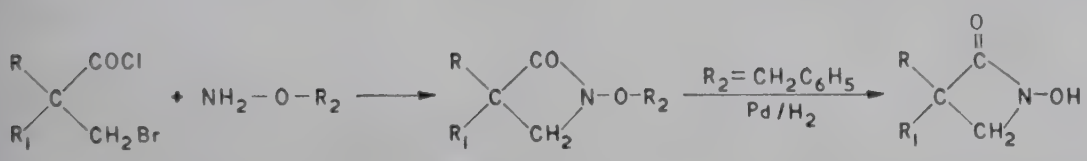
1-methyl-3,3-di-n-propylazetidine-2-one
L 3432

Continuing our chemical studies, it was observed that condensation of only slightly basic amines with the chlorides of β-propionic acids leads directly to azetidin-2-ones substituted at the nitrogen. Thus, *o*-nitroaniline condenses with the chlorides of β-bromopropionic acid (I) forming the azetidinones (II) which can be reduced with Pd H_2 (azetidin-2-ones are stable in these conditions) into 1-(*o*-aminophenyl)-azetidin-2-ones (III). The observation that the action of dilute acids leads to rearrangement of these compounds is rather astonishing. A new class of compounds — 3,3-disubstituted 2,3,4,5-tetrahydro-1H-1,5-benzodiazepine-2-ones (IV)[47] is thus obtained. The same products are given by cyclization of *o*-phenylendiamine with chlorides of α-substituted β-bromopropionic acids subsequently treated with sodamide in liquid ammonia.

Another interesting example of the reaction of β-bromopropionic acid chlorides with only slightly basic amines is their condensation with some *o*-alkyl- or *o*-aryl-hydroxylamines[48] leading to N-alkoxy- or N-aryloxy-azetidin-2-ones. Using hydrogen and palladium, it is possible to catalytically debenzylate N-benzyloxyazetidin-2-ones obtaining, in practically quantitative yields, the N-hydroxyazetidin-2-ones which have been characterized as the sodium salts. N-hydroxyazetidin-2-ones, which are, in reality, cyclic hydroxamic acids, have no activity on the central nervous system, just like



their numerous derivatives also prepared during the systematic chemical study.



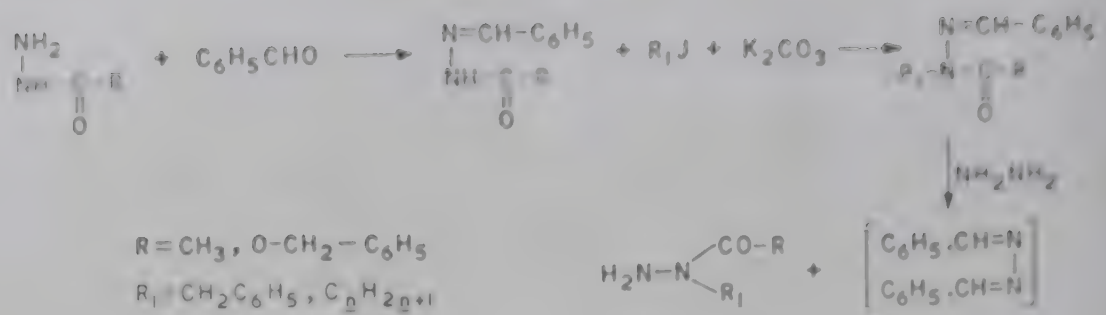
$R_2 = C_6H_5CH_2$; $C_6H_5(CH_2)_2$; CH_2CH_2OH, C_2H_5, n-C_3H_7, i-C_3H_7, n-C_4H_9, CH_3

Perfecting of the process for preparing azetidin-2-ones through condensation of the β-bromopropionamides using sodamide in liquid ammonia, has supplied a general method for the synthesis of 1-amino-azetidin-2-ones and their derivatives[49].

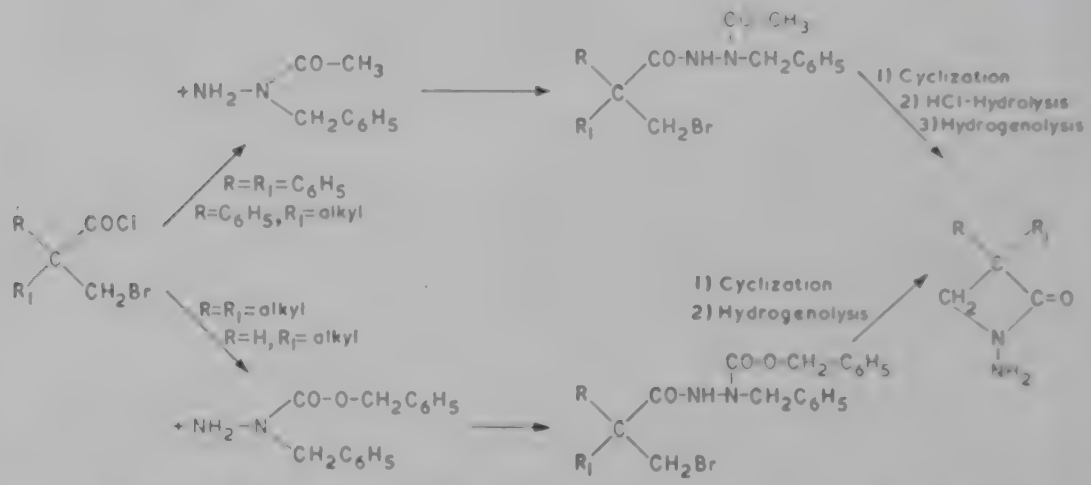
Very little mention is made about these compounds in the chemical literature. As far as we are aware, only three derivatives (1-dimethylamino-3-methylazetidin-3-one, 1-dimethylamino-4-methylazetidin-2-one and 1-methylaminoazetidin-2-one) and their corresponding methiodides have been recently described by Russian investigators[50,51].

We, therefore, first synthesized the N-acyl-N-benzylhydrazines, which were obtained with a new procedure perfected in our laboratories[52]. It consists of allowing the known *N*-acylhydrazines to react with benzaldehyde, successively alkylating the benzylidene derivative according to a previously described technique (K_2CO_3, acetone, RI)[53,54]. Finally, the Schiff's base undergoes hydrazinolysis thus giving rise to high yields of the required *N*-acyl-*N*-alkylhydrazines. Very little information is available on these compounds in the chemical literature.

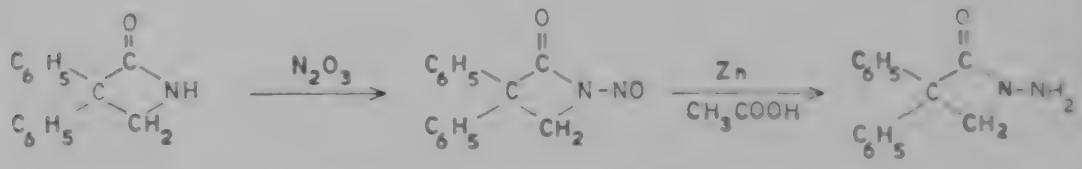
The *N*-acyl-*N*-alkylhydrazines are condensed with the chlorides of α-substituted β-bromopropionic acids, and the amides obtained cyclized with sodamide in liquid ammonia. The 1-(*N*-acyl-*N*-alkyl)-aminoazetidin-2-ones are regularly obtained. In the case of the 1-(*N*-acetyl-*N*-benzyl)-3,



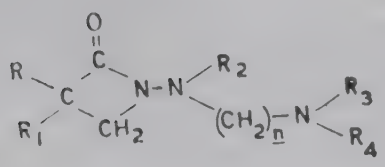
3-diphenyl and 3-phenyl-3-alkyl derivatives, it is possible to saponify the acetyl group by means of dilute mineral acids, finally catalytically debenzylating (hydrogenolysis) the intermediate 1-(N-benzyl)-aminoazetidin-2-one. In the case of 3, 3-dialkyl and 3-monoalkyl derivatives this procedure is unsuitable as acid hydrolysis leads to molecular rearrangement as described later on. Here it is best to use N-carbobenzyloxy-N-benzylhydrazine so as to avoid the use of acid, obtaining the 1-amino-azetidin-2-ones by simple hydrogenolysis.



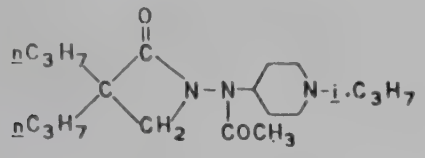
1-Amino-3, 3-diphenylazetidinone has also been prepared by a different process consisting of allowing the 3, 3-diphenylazetidin-2-one itself to react with nitrous gas, subsequently reducing the N-nitroso derivative with zinc and acetic acid. This reduction cannot be performed on the N-nitroso derivatives of azetidin-2-ones with other substituents at C_3.



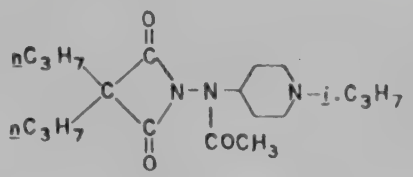
Using suitable hydrazine derivatives, even with basic substituents, it has been possible to obtain a large number of compounds possessing the general formula:



These products and numerous derivatives have also been screened, but no specially interesting pharmacological property has emerged. In particular we have been surprised at the absence of any anti-inflammatory activity at the level of compounds (I), 1-[*N*-(1-isopropyl-4-piperidyl)-*N*-acetylamino]-3, 3-dipropylazetidin-2-one, when compared with compound (II), which was found by Ebnother *et al.*[55] to be ten times more active than butasolidine as antiphlogistic in the formalin oedema test.

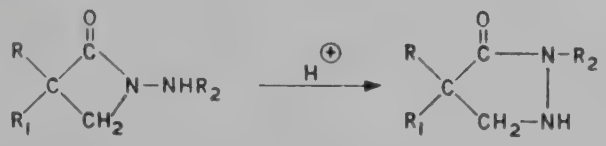


(I)



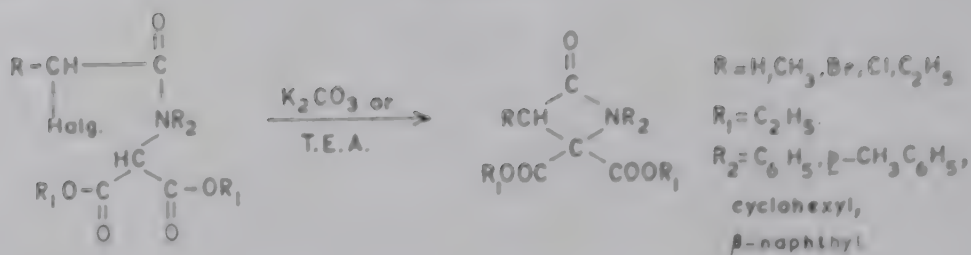
(II)

The 1-aminoazetidin-2-ones are unstable in acid medium and the instability progressively increases on passing from the 3, 3-diphenyl, to the 3-phenyl-3-alkyl, 3, 3-dialkyl and 3-mono-alkyl derivatives, being maximum in the last. The compounds, whether the amine group carries no substituent or is alkylated, transpose, with excellent yields, into 4-mono and 4,4-disubstituted pyrazolidine-3-ones. We have carried out a thorough study[56,57] on this class of compounds, which can also be directly reached from β-bromopropionic acids and their derivatives.

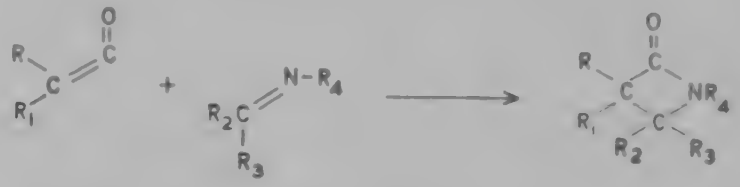


Apart from the three methods for synthesizing azetidin-2-ones described, other single preparative techniques suitable for the synthesis of special structures have been described by various authors. It may be pointed out that none of these methods, apart from those given later on is suitable for the synthesis of β-lactams non-substituted at the nitrogen. Moreover, no product thus obtained appears to have been submitted to biological screening. The most significant processes are as follows.

Synthesis by intramolecular alkylation of an α-haloacylaminomalonic ester. This process, described by Sheehan and Bose in 1950[58] has permitted the obtaining of a good number of *N*-alkyl or *N*-arylazetidin-2-ones[59–61]. Yields were good and a number of chemical transformations on the heterocyclic compounds were also carried out.

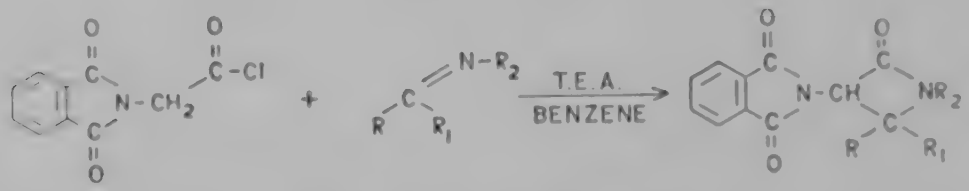


Synthesis by cyclo-addition of ketenes and azomethines. This process is of great historical importance as Staudinger[3] used it in 1907 to synthesize the first azetidinone. The condensation reaction of a ketene[65] with a Schiff's base (azomethine) is one of a very general process that is conveniently called cyclo-addition.



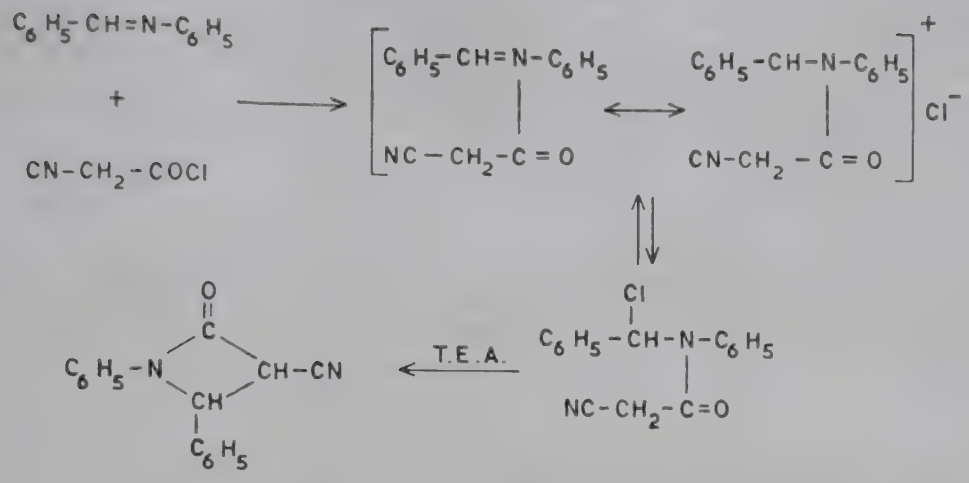
A large number of β-lactams have been obtained with this process by Staudinger himself[66–72], Kirmse and Horner[73], Pfleger and Jäger[74], and other authors[75,76].

Synthesis by addition of acid chlorides to azomethines. This process, which has permitted the obtaining of some 3-acyl-aminoazetidin-2-ones, has been perfected by Sheehan and Ryan[77,78] within the framework of studies aimed at the total synthesis of penicillin. The possibilities of its use appear to be limited and a correlation with Staudinger's process is obvious.

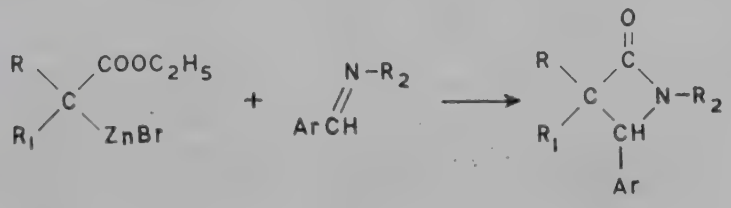


Recently, Boehme *et al.*[79] have used this technique, carrying out condensation of cyanoacetyl chloride with a Schiff's base ($C_6H_5CH=N-C_6H_5$). The reaction product treated with triethylamine gives 1, 4-diphenyl-3-cyanoazetidin-2-one.

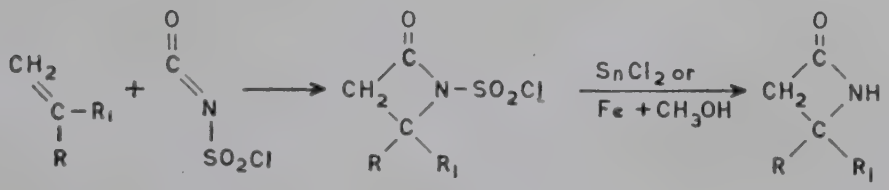
Synthesis by reacting α-bromozinc ester with azomethines. The process was described in 1943 by Gilman and Speeter[80] and subsequently used by other investigators[18,81,82] for obtaining eight azetidin-2-ones. According to Moore[8] (p. 937) "...the condensation doubtless proceeds by nucleophilic attack of the organozinc compound at the -C=N bond.



forming the bromozinc complex of the amine anion which is also an intermediate in the cyclization of β-amino esters with Grignard reagents".

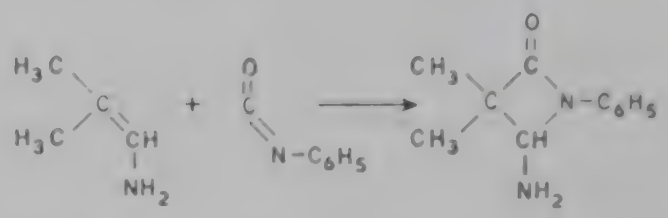


Synthesis by cyclo-addition of isocyanates and olefines. This reaction was first described by Graf (Farbwerke Hoechst)[83-85] who condensed chlorosulphonylisocyanate with a series of olefines. 4, 4-Dialkyl (or 4-phenyl)-1-chlorosulphonylazetidin-2-ones were obtained. The 1-chlorosulphonyl group may be removed by reduction (Fe powder in CH_3OH or with $SnCl_2.2H_2O$) without breaking of the heterocyclic structure. This process is therefore suitable for synthesis of 4-substituted azetidin-2-ones. The compounds which we obtained also by another route[22], were submitted to pharmacological screening, but no result worthy of notice has emerged.



Synthesis by condensation of phenylisocyanate with N, N-disubstituted isobutanylamines. This process, developed during the studies on the chemistry of enamines, has found only very limited use, at

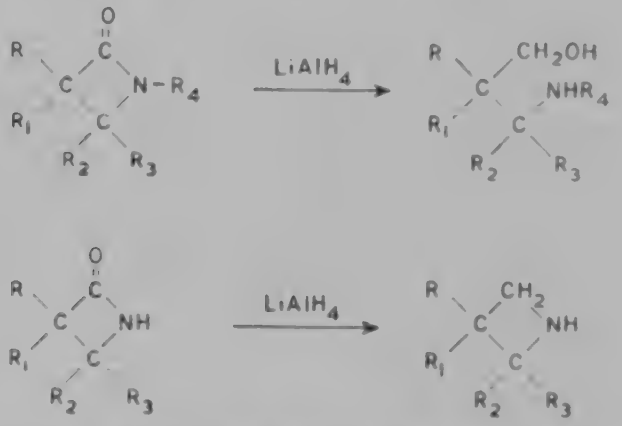
least at present. A first use has been described by Perelman and Mizsak[87] leading to 4-amino-3, 3-dimethyl-1-phenylazetidin-2-one. Other β-lactams have been subsequently obtained with this technique by Opitz[86] and Bose[88].



Finally, the example described by Sheehan and Izzo[89] is worth mentioning; here, phenylisocyanate reacts with diazomethane, giving rise to the formation of 1-phenylazetidin-2-one. Use of this technique appears to be limited to this single case.

Faced with this relative abundance of synthetic routes, we observed great scarcity of information relative to the possibility of chemically transforming the azetidin-2-ones. Three reactions, all breaking the heterocyclic structure, are essentially known in the literature.

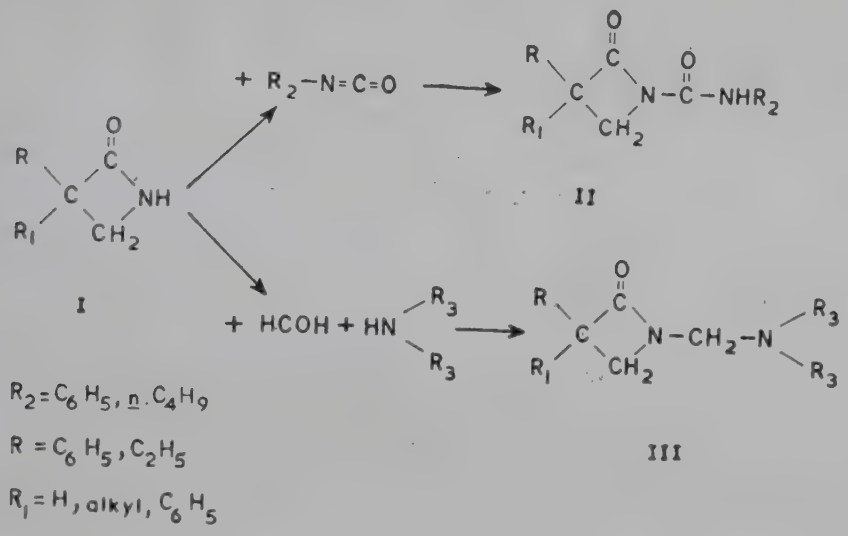
1) Azetidinones are usually unstable if heated at high temperature. Staudinger himself observed[65] retrocyclo-addition in this case with formation of ketenes and ketones, the latter originating from the azomethines.



2. 1-Substituted β-lactams are reduced by $LiAlH_4$, with consequent breaking of the heterocyclic compounds and formation of γ-substituted aminopropanol derivatives[13 90 91]. The same phenomenon occurs also in the case of 1-substituted azetidin-2, 4-diones[92]. By contrast, we have ascertained that 1-unsubstituted azetidin-2-ones are reduced by means of $LiAlH_4$ into the corresponding azetidines (or trimethyleneimines)[91]. This observation has opened the field to systematic chemical and pharmacological study of this class of compounds, performed in our laboratories[93] and still in progress.

3) The reaction of azetidin-2-ones which has been most studied is their hydrolysis in acid and alkaline medium, the course of which strictly depends on the type and position of the substituents, and usually gives rise to β-aminopropionic acid derivatives[8,11,37]. As can be seen, all these reactions prejudice the existence of the β-lactam structure.

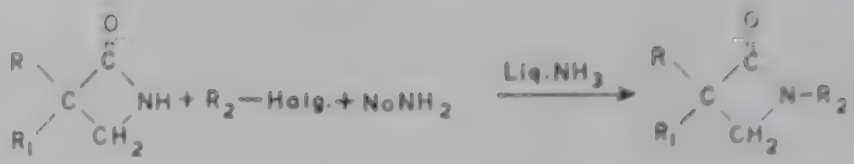
A first non-demolitory reaction has been described by Cignarella *et al.*[29]: the C_3-substituted azetidin-3-ones (I) react with phenylisocyanate and *n*-butylisocyanate) giving carbomylazetidin-2-ones (II), whose structure has been demonstrated. Moreover, β-lactams react with aqueous formaldehyde and secondary amines[27,29], such as pyrollidine, morpholine, diethylamine and 3, 3-dimethylazetidine[91], giving the corresponding Mannich' bases[93].



Preparation of 1-alkyl substituted azetidin-2-ones through cyclization of β-bromo-*N*-alkylpropionamide with sodamide in liquid ammonia is very easy. By contrast the synthesis of the same β-lactams by reaction of the Grignard reagent with the *N*-monosubstituted β-aminopropionic esters present great difficulties in obtaining the necessary intermediate[29].

In a single case[27] was *N*-methylation of the β-lactam obtained, that is, for 3-phenyl-3-hydroxymethylazetidin-2-one, using conventional techniques [$(CH_3)_2SO_4$ in alkaline medium]. Finally, alkylation in position I was carried out by allowing the 3-substituted azetidin-2-ones to react with stoichiometric quantities of sodamide and alkylhalogenides in the presence of liquid ammonia. A certain number of *N*-alkylazetidin-2-ones have thus been obtained[46].

During this systematic study[91], it has been possible to determine also suitable experimental conditions for *N*-acylation, *N*-hydroxymethylation and *N*-dialkylamino-alkylation of azetidin-2-ones. Finally, 1-vinyl-3, 3-di-

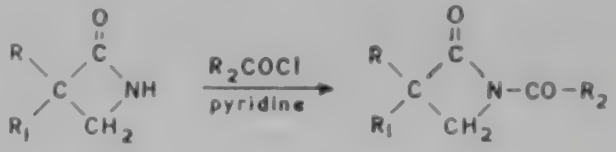


$R = C_nH_{2n+1}, C_6H_5, C_6H_4CH_3\underline{p}, C_6H_4OCH_3\underline{p}$.

$R_1 = H, C_nH_{2n+1}, C_6H_5$

$R_2 = C_nH_{2n+1}$

n-propylazetidin-2-one was synthesized. 3-Substituted azetidin-2-ones react in pyridine solution with acid chlorides both aliphatic and aromatic on warming. The structure of *N*-acyl β-lactams has been spectrophotometrically (I. R.) confirmed.

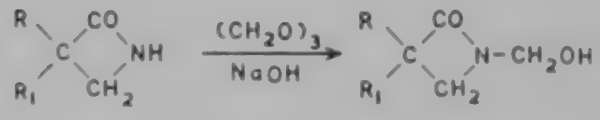


Ia,b IIa,b

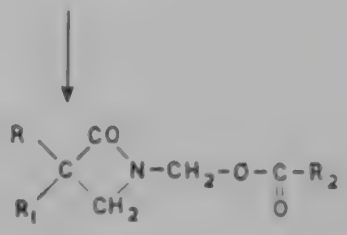
a: $R = R_1 = \underline{n}C_3H_7$ b: $R = C_6H_5, R_1 = C_2H_5$

$R_2 = C_2H_5, \underline{p}ClC_6H_4$

Beta-lactams react in basic alcoholic media with trioxymethylene, giving rise to *N*-hydroxymethyl derivatives. The latter, whose structure



Ia,b IIa,b



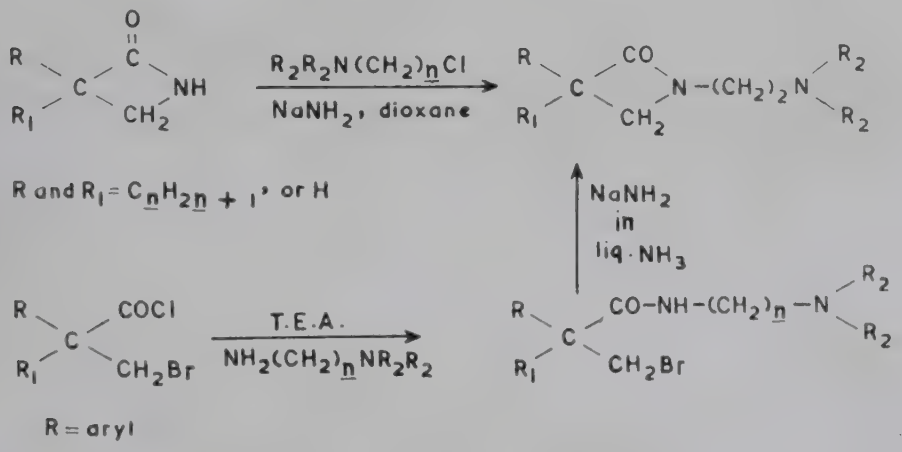
IIIa,b

IIIa $R_2 = C_2H_5$; CH_2Cl; C_6H_5; $\underline{p}ClC_6H_4$; 3,4,5-$(CH_3O)_3C_6H_2$.

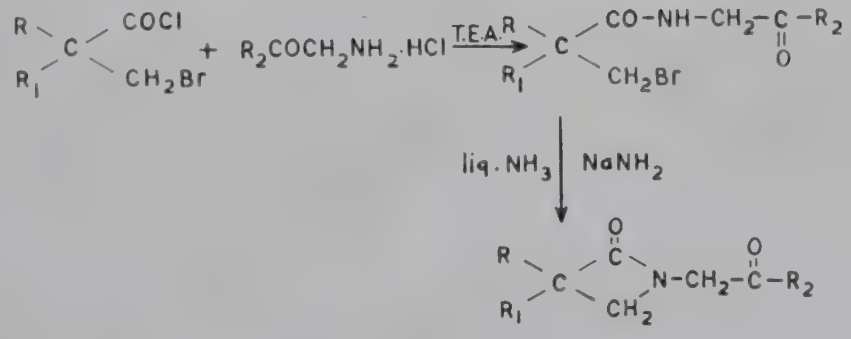
IIIb: $R_2 = C_2H_5$; C_6H_5; $\underline{p}ClC_6H_4$; 3,4,5-$(CH_3O)_3C_6H_2$.

has been confirmed by I. R. spectra, can be condensed with acid chlorides, in the presence of triethylamine, thus obtaining *N*-acyloxymethylazetidin-2-ones.

Condensation of azetidin-2-ones with 2-chloroethyl-(or 3-chloropropyl)-dialkylamines, on heating in dioxane solution and in the presence of sodamide, can be performed only with 3, 3-di-(or 3-mono)-alkyl substituted β-lactams. The presence of an aryl radical at C_3 leads, as might be expected[23,24] only to polymerization products. In this case (3-aryl substituted derivatives), synthesis of the desired compounds may be easily carried out by condensing 2-bromo-*N*-(*N'*-alkylaminoalkyl)-propionamides with sodamide in liquid ammonia[46].

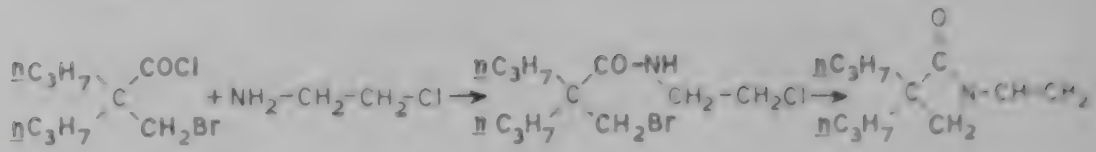


Direct alkylation of azetidin-2-ones, whatever the substituent at C_3, cannot be performed with α-haloketones. Consequently, also in this case, it was necessary to utilize cyclization of the β-halopropionamides obtained by condensation of β-bromopropionic acid chlorides with the arylaminomethyl-ketones.



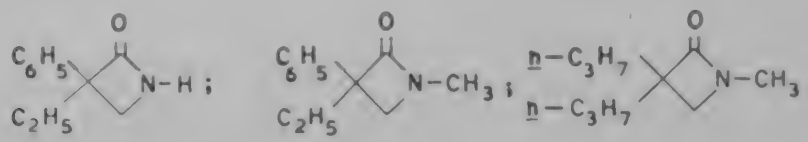
A peculiar and unexpected result was obtained during cyclization of *N*-(β-chloroethyl)-α, α-di-*n*-propyl-β-bromopropionamide. Due to simultaneous intramolecular dehydrohalogenation, 1-vinyl-3, 3-di-*n*-propylazeti-

dine-2-one is obtained, which is somewhat an unstable oil spontaneously tending to polymerize.



Pharmacology of the Azetidin-2-ones

An equally systematic pharmacological screening was performed in our laboratory parallel to the chemical study of azetidin-2-ones. During this screening, three compounds were found to be of particular interest for their activity on the central nervous system, and these are dealt with in detail. In fact, they are still the best derivatives obtained in the single series as regards biological behaviour and are already the result of a thorough chemical study. Obviously, this does not exclude that other better or different products may be identified in the future. The pharmacological results will be briefly described in order to give some example of the pharmacological properties which are the most interesting and more frequently found among azetidinone derivatives. The three compounds of peculiar interest have the following chemical structures:



L 1657	L 1743	L 3432
3-phenyl-3-ethyl-azetidin-2-one	1-Methyl-3-phenyl-3-ethylazetidin-2-one	1-Methyl-3-di-n-propylazetidin-2-one

3-Phenyl-3-ethylazetidin-2-one (L 1657). This derivative produces sedative effects in mice, rats and dogs which, with increase of the dose, are accompanied by ataxia and impairment of the righting reflex. The LD_{50} i.p. in mice is 450 mg/kg. In rats, L 1657 was compared with phenobarbital and 2-ethyl-2-phenylglutarimide for its ability to shorten the time necessary to regain the sleeping posture after abrupt awakening. According to this technique, described by Maffii[93], which proved to be of value for detecting the activity of known hypnotic substances, L 1657 appeared somewhat less active than phenobarbital, and almost equally effective as 2-ethyl-2-phenylglutarimide (Doriden®—Ciba). Moreover, L 1657 synchronizes the cortical electrical activity in rabbits, cats, and dogs, and selectively depresses polysynaptic reflexes in cats[17]. L 1657 protects mice from maximal electroshock and pentylenetetrazol seizures. The profile of L 1657's sedative-

TABLE 1—ACTION ON CONDITIONED AVOIDANCE BEHAVIOUR

Compound	Administration route	ED_{50} (mg./kg.) and C.L.					
		CR_2*		CR†		UR‡	
L 1657	i.p.	142	(111-182)	231	(199-268)	231	(199-268)
L 1743	i.p.	37	(21-65)	170	(142-202)	170	(142-202)
L 3432 (susp.)	i.p.	12·7		14·9		69	
L 3432 (sol.)	i.p.	8		19·6		69	
L 3432 (sol.)	oral	15		23		111	
Barbital Na	oral	85	(53-137)	112	(83-151)	134	(102-175)
Meprobamate	i.p.	32·5	(13·0-81·2)	170	(139-207)	170	(139-207)
Meprobamate	oral	163		475		475	
Chlorpromazine	oral	1·75	(0·9-3·3)	11·6	(9·0-14·7)	33	(25·4-42·9)
Chlordiazepoxide	i.p.	7·65		15		15	
Chlordiazepoxide	oral	17·4		36·8		70	

*Secondary conditioned response
†Primary conditioned (avoidance) response
‡Unconditioned (escape) response

hypnotic activity received further confirmation from studies on conditioned rats[96]. The results of these studies are given in Table 1 which show the doses abolishing the secondary and primary conditioned responses and the unconditioned response in 50 per cent of the treated animals, in comparison with known hypnotics and tranquillizers. It can be seen that the activity of L 1657 appears substantially similar to that of barbital sodium, from a qualitative point of view. Studies on chronic toxicity in animals, especially dogs, gave unfavourable results, while the results obtained in clinical trials confirmed the laboratory findings as far as the sedative and hypnotic activities are concerned.

1-Methyl-3-phenyl-3-ethylazetidin-2-one (L 1743). As regards effects on animal behaviour, this compound appears different from L 1657. Its LD_{50} i.p. in mice is 580 mg./kg. In conditioned animals only (Table 1) its activity appears very similar to that of meprobamate: it inhibits the secondary conditioned response in doses more than four times less than those blocking the primary conditioned and the unconditioned responses[18]. In mice and rats L 1743 showed muscle relaxant activity and its ability to

tame and quieten aggressive animals was demonstrated in different animal species. From the above described results, L 1743 appears to have, at least in animals, the pharmacological effects of so-called minor tranquillizers, such as meprobamate and chlordiazepoxide (the former appearing also quantitatively identical).

Sedative hypnotic and anticonvulsant actions of a series of azetidinone derivatives, including L 1657 and L 1743, were reported in 1959 by Maffii[9].

It can be seen from the results of animal studies described above that methylation of L 1657 in position one leads to decrease of neurotoxic effects and increase of behavioral specificity, thus passing from a hypnotic to a tranquillizing effect.

1-Methyl-3, 3-di-N-propylazetidin-2-one (L 3432). This phenomenon is even more pronounced for this compound, in which the ethyl and phenyl groups of L 1743 are substituted by two *n*-propyl groups. The compound no longer produces ataxia and impairment of the righting reflex and it inhibits specifically the conditioned avoidance behaviour (Table 1). However, it differs from known drugs because of the relationship between the different response-blocking doses; the same dose inhibits both the secondary and primary conditioned responses without affecting the unconditioned response. Its LD_{50} i.p. in mice is 321 mg./kg.

Moreover, L 3432 shows a characteristic diphasic action in rats, cats and dogs: an excitatory phase immediately after treatment, followed by sedation. The initial excitatory phase is missing in monkeys. Among the different animal species, rats and monkeys seem to be the most sensitive to the sedative action of L 3432. The compound potentiates barbiturate narcosis in mice and antagonizes the cataleptic action of chlorpromazine and reserpine ptosis in the same animal species.

Compound L 3432 is at present under clinical trial and preliminary results seem to indicate its good tolerability, and therapeutic activity on psychotic patients.

CONCLUSION

It is an undoubted fact that azetidin-2-ones, variously substituted at C_3 and at the nitrogen, form a class of compounds which are definitely and significantly active on the central nervous system. This activity may assume various aspects and pass from the plain sedative action to various kinds of hypnotic and tranquillizing effects. It is closely linked with and dependent on the heterocyclic structure. In fact, hydrolysis products (β-aminopropionic acid derivatives) are completely free from activity. The preliminary results of clinical trials confirm that the activity observed in laboratory animals can be transferred to the human field.

Our future goal will then naturally consist not only in more thoroughly studying and extending chemical and pharmacological knowledge of the class but also in preparing products of therapeutic value.

REFERENCES

1. Gross, F., Tripod, J. & Meier, R., *Schweiz. med. Wschr.*, **85** (1955), 305.
2. Dalmer, O., Diehl, C. & Pieper, H., USA Patent, n. 2,259,925 (21-12-1942).
3. Goodman, L. S., Swinyard, E. A., Brown, W. C., Schiffman, D. O., Grewal, M. S. & Bliss, E. L., *J. Pharmac. exp. Ther.*, **108** (1953), 428.
4. Ferrari, V., *Il Farmaco*, **9** (1954), 211.
5. Staudinger, H., *Ann.*, **356** (1907), 51.
6. Breckpot, R., *Bull. Soc. chim. Belg.*, **32** (1923), 412.
7. Sheehan, J. C. & Henery-Logan, K. R., *J. Am. chem. Soc.*, **81** (1959), 3089.
8. Moore, J. A., Heterocyclic Compounds with Three and Four-membered Ring: Pt. II (ed. A. Weissberger) New York, 1964, p. 885.
9. Holley, R. W. & Holley, A. D., *J. Am. chem. Soc.*, **71** (1949), 2129.
10. Adams, R. & Jones, V. V., *J. Am. chem. Soc.*, **71** (1949), 3826.
11. Testa, E., Fontanella, L. & Fava, F., *Il Farmaco.*, **13** (1958), 152.
12. Fischer, E., *Berichte*, **38** (1905), 2914.
13. Blicke, F. F. & Gould, W. A., *J. org. Chem.*, **23** (1958), 1102.
14. Testa, E., Fava, F. & Fontanella, L., *Liebigs Annln. Chem.*, **614** (1958), 167.
15. Testa, E. & Fontanella, L., *Liebigs Annln. Chem.*, **622** (1959), 117.
16. Maffii, G., *Il Farmaco*, **14** (1959), 176.
17. Maffii, G., Silvestrini, B. & Bianchi, G., *Il Farmaco*, **14** (1959), 269.
18. Maffii, G., *Il Farmaco*, **14** (1959), 383.
19. Testa, E., Fontanella, L., Cristiani, G. F. & Fava, F., *Liebigs Annln. Chem.*, **614** (1958), 158.
20. Testa, E. & Fontanella, L., *Liebigs Annln. Chem.*, **625** (1959), 95.
21. Testa, E., Fontanella, L. & Aresi, V., *Liebigs Annln. Chem.*, **656** (1962), 114.
22. Testa, E., Fontanella, L. & Aresi, V., *Liebigs Annln. Chem.*, **673** (1964), 60.
23. Testa, E. & Fontanella, L., *Liebigs Annln. Chem.*, **625** (1959), 95.
24. Graf, R., Lohaus, G., Borner, K., Schmidt, E. & Bestian, H., *Ang. Chem.* **74** (1962), 523.
25. Bonati, A., Cristiani, G. F. & Testa, E., *Liebigs Annln. Chem.*, **647** (1961), 83.
26. Testa, E., Bonati, A., Pagani, G. & Gatti, E., *Liebigs Annln. Chem.*, **647** (1961), 92.
27. Testa, E. & Fontanella, L., *Liebigs Annln. Chem.*, **661** (1963), 187.
28. Testa, E. & Fontanella, L., *Liebigs Annln. Chem.*, **671** (1964), 106.
29. Cignarella, G., Cristiani, G. F. & Testa, E., *Liebigs Annln. Chem.*, **661** (1963), 181.
30. Fontanella, L. & Testa, E., *Liebigs Annln. Chem.*, **616** (1958), 148.
31. D'Amato, V., Peliza, G., Bersanelli, G. & Testa, E., *Liebigs Annln. Chem.*, **635** (1960), 127.
32. Testa, E., Fontanella, L., Cristiani, G. F. & Mariani, L., *Helv. chim. Acta.*, **42** (1959), 2370.
33. Testa, E. & Fontanella, L., *Liebigs Annln. Chem.*, **660** (1962), 118.
34. Cignarella, G., Mariani, L. & Testa, E., *Gazz. chim. Ital.*, **95** (1965), 831.
35. Testa, E., Fontanella, L., Cristiani, G. F. & Fava, F., *Liebigs Annln. Chem.*, **619** (1958), 47.
36. Testa, E., Fontanella, L., Cristiani, G. F. & Mariani, L., *Liebigs Annln. Chem.*, **639** (1961), 166.
37. Testa, E., Fontanella, L., Cristiani, G. F. & Gallo, G. G., *J. org. Chem.*, **24** (1959), 1928.
38. Zaugg, H. E., *Org. Reactions*, (1954), 307.
39. Sheehan, J. C., Hasspacher, K. & Lieh Yeh, Y., *J. Am. chem. Soc.*, **81** (1959), 6086.
40. Testa, E., Fontanella, L. & Mariani, L., *J. org. Chem.*, **25** (1960), 1812.
41. Ludwig, B. J., *J. Am. chem. Soc.*, **72** (1950), 5329.
42. Nicolaus, B. J. R., Mariani, L. & Testa, E., *Helv. chim. Acta.*, **44** (1961), 1076.

43. Knunyants, I. L. & Gambaryan, N. P., *Izvest. Akad. Nauk. S.S.S.R., Otdel Khim. Nauk*, (1955), 1037 [C.A., **50** (1956), 11127].
44. Knunyants, I. L. & Gambaryan, N. P., *Izvest. Akad. Nauk S.S.S.R. Otdel Khim. Nauk*, 1957, 834 [C.A., **52** (1958), 3816].
45. Knunyants, I. L., Rytslin, E. E. & Gambaryan, N. P., *Izvest. Akad. Nauk S.S.S.R Otdel Khim. Nauk*, (1961), 183 [C.A., **55** (1961), 18696].
46. Testa, E., Nicolaus, B. J. R., Bellasio, E. & Mariani, L., *Liebigs Annln. Chem.*, **673** (1964), 71.
47. Nicolaus, B. J. R., Bellasio, E., Pagani, G., Mariani, L. & Testa, E., *Helv. chim. Acta* (in press).
48. Nicolaus, B. J. R., Bellasio, E., Pagani, G. & Testa, E., *Gazz. chim. Ital.*, **93** (1963), 648.
49. Pifferi, G., Consonni, P. & Testa, E., *Gazz. chim. Ital.*, (in press).
50. Sokolova, T. A. & Ovsyannikova, L. A., *Dokl. Akad. Nauk. S.S.S.R.*, **143** (1), (1962), 140; [C.A., **57** (1962), 4616].
51. Zapevalova, N. P., Sokolova, T. A., Bazhena, N. M. & Kaltsov, A. J., *Dokl. Akad. Nauk S.S.S.R.*, **150** (3), (1963), 551; [C.A., **59** (1963), 7454].
52. Cignerella, G., *J. org. Chem.*, (in press).
53. Davies, J. S. H. & Hook, W. H., *J. chem. Soc.*, (London), (1950), 30.
54. Testa, E. & Ettorre, R., *Ark. der Pharmazie*, **290** (1957), 552.
55. Ebnother, A., Jucker, E., Rissi, E., Butschmann, J., Schreier, E., Stiner, R., Suss, R. & Vogel, A., *Helv. chim. Acta*, **42** (1959), 918.
56. Nicolaus, B. J. R., Mariani, L. & Testa, E., *Helv. chim. Acta*, **44** (1961), 2059.
57. Nicolaus, B. J. R., Mariani, L., Bellasio, E. & Testa, E., *Gazz. Chim. Ital.*, **94** (1964), 652.
58. Sheehan, J. C. & Bose, A. K., *J. Am. chem. Soc.*, **72** (1950), 5158.
59. Sheehan, J. C. & Bose, A. K., *J. Am. chem. Soc.*, **73** (1951), 1761.
60. Bose, A. K., Ghosh-Mazumdar, B. N. & Chatterjee, B. G., *J. Am. chem. Soc.*, **82** (1960), 2382.
61. Bose, A. K. & Manhas, M. S., *J. org. Chem.*, **27** (1962), 1244.
62. Bose, A. K., Manhas, M. S. & Ghosh-Mazumdar, B. N., *J. org. chem.*, **27** (1962), 1458.
63. Chatterjee, B. G., Moza, P. N. & Roy, S. K., *J. org. Chem.*, **28** (1963), 1418.
64. Bose, A. K., Manhas, M. S. & Ramer, R. M., *Abstr. A.*, *19th int. Congr. of Pure and appl. Chem.*, (1963), 208.
65. Staudinger, H., *Die Ketene*, *Enke*, Stuttgart (1912).
66. Staudinger, H., *Berichte*, **40** (1907), 1145.
67. Staudinger, H., *Berichte*, **50** (1917), 1035.
68. Staudinger, H. & Jalagin, S., *Berichte*, **44** (1911), 365.
69. Staudinger,, H. Klever, H. W. & Kober, P., *Liebigs Annln. Chem.*, **374** (1910), 1.
70. Staudinger, H. & Klever, H. W., *Berichte*, **40** (1907), 1149.
71. Staudinger, H. & Maier, J., *Liebigs Annln. Chem.*, **401** (1913), 292.
72. Staudinger, H. & Ruzicka, L., *Liebigs Annln. Chem.*, **380** (1911), 278.
73. Kirmse, W. & Horner, L., *Berichte*, **89** (1956), 2759.
74. Pfleger, R. & Jager, A., *Berichte*, **90** (1957), 2460.
75. Holley, A. D. & Holley, R. W., *J. Am. chem. Soc.*, **73** (1951), 3172.
76. Van Leusen, A. M. & Arens, J. F., *Rec. Trav. Chim.*, **78** (1959), 551.
77. Sheehan, J. C. & Ryan, J. J., *J. Am. chem. Soc.*, **73** (1951), 1204.
78. Sheehan, J. C. & Ryan, J. J., *J. Am. chem. Soc.*, **73** (1951), 4367.
79. Boehme, H., Ebel, S. & Hartke, K., *Berichte*, **98** (1965), 1463.
80. Gilman, H. & Speeter, M. A., *J. Am. chem. Soc.*, **65** (1943), 2255.
81. Gould, W. A., Ph.D. Thesis, University of Michigan (1959).
82. Miller, R. E. & Nord, F. F., *J. org. Chem.*, **16** (1951), 1720.
83. Graf, R., German Patent 941,487 (19.12.1953) to Farbwerke Hoechst A. G. (C. 1957, 1927).

84. Farbwerke Hoechst, A. G., Belg. Patent n. 577,006 (23.3.1959).
85. Graf, R., *Liebigs Annln. Chem.*, **661** (1963), 111.
86. Perelman, M. & Mizsak, S. A., *J. Am. chem. Soc.*, **84** (1962), 4988.
87. Optiz, G. & Koch, J., *Ang. Chem.* (int. ed.), **2** (1963), 152.
88. Bose, A. K. & Mina, G. L., *Abstr. Paper*, 148*th Meet. ACCS.*, (1964), 18.
89. Sheehan, J. C. & Izzo, P. T., *J. Am. chem. Soc.*, **71** (1949), 4059.
90. Speeter, M. E. & Maroney, W. H., *J. Am. chem. Soc.*, **76** (1954), 5810.
91. Testa, E., Fontanella, L. & Cristiani, G. F., *Liebigs Annln. Chem.*, **626** (1959), 114.
92. Testa, E., Wittgens, A., Maffii, G. & Bianchi, G., Research Progress in Organic-biological and Medicinal Chemistry, Vol. I, Societa Editoriale Farmaceutica—Milano—1964, p. 477.
93. Reichert, B., Die Mannich—Reaktion, Springer Verlag, Berlin, 1959.
94. Testa, E., Piffari, G., Fontanella, L. & Aresi, V., *Liebigs Annln. Chem.* (in press).
95. Maffii, G., *Il Farmaco*, **13** (1958), 581.
96. Maffii, G., *J. Pharm. Pharmacol.*, **11** (1959), 129.

Pharmacology of Nitrogen-free Bitter Principles of *Lactuca virosa*: Destruction of these Substances During Extraction and Drying

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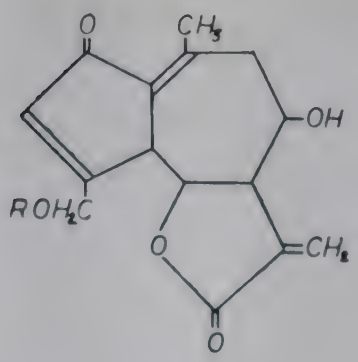
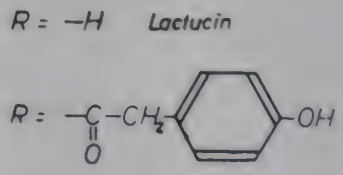
In 1935, my academic teacher, Benno Bleyer, gave me the suggestion to carry out chemopharmaceutical investigations on *Lactuca virosa*. Under the name 'opium frigidum', the dried latex of *Lactuca virosa*, the lactucarium, once played a very important role in the treatment of irritable cough and as a sedative. A nitrogen-free bitter substance, lactucin, was isolated 100 years ago. Working with the author, the Munich pharmacologist Forst discovered a second bitter principle, lactucopikrin, also free of nitrogen. The formulae of these substances were at that time unknown but they became of interest when Forst discovered that both developed central sedative effects with very low toxicity. We now know the formulae; they were found by Sorm and, independently, by Barton. The substances have structures similar to azulene.





Lactucopikrin

According to the investigations of Forst, the effect of lactucarium on mice is as follows: The animals initially remain seated for a certain time, then crouch sleepily in their place, a state which can continue for hours. The drowziness often lasts until the next day. Hyperaemia of the tail is frequently observed. With fatal doses, central paralysis is more pronounced; the animal begins to reel, assumes a position on its stomach and later on its side. Reflexes disappear after 1 to 2 hr. On the following day, it is frequently possible to observe an increase in reflex excitability; it is possible that temporary clonic spasm may occur. The same effect is observed after dosage with either of the pure substances.

On intravenous injection of sub-lethal doses of galenical preparations or of either of the pure substances, in experiments carried out on rabbits, the blood pressure and respiration are not affected.

The behaviour of the animals proves that lactucarium as well as the two pure substances extracted, lactucin and lactucopikrin, definitely produce central paralysis.

After this discovery, the primary aim of subsequent pharmacological examinations was to support chemopharmaceutical investigations to provide basic information relevant to problems involving the time of extraction of the milky juice, its separation, its further treatment and its storage stability. The aim of this work was to determine the most effective preparation. The sedative effect was chosen as a criterion of effectiveness.

The sedative effect is the first and the most sensitive stage of a narcotic effect and its determination from experiments carried out on animals involves great difficulties.

In this connection, lactucarium offers a certain advantage since its central paralysing influence even at the maximum permissible dosage always remains within the limits of a sedative effect and since, in the interpretation of results, the danger of an unnoticed transition into the region of narcotics, which would complicate experiments considerably does not exist.

The experimental characterization of this most sensitive stage of central tranquillization required a new sensitive method of approach which, however, should not in the least be associated with other influences on the animal employed for the experiments. The method evolved by Forst seemed to provide the maximum protection since the only operation carried out on the animal involved the single act of treatment with the substance either by injection or by stomach tube. The degree to which the alertness of the animal is repressed, is registered automatically by the animal itself. The manifestation of the sedative effect is recorded on the basis of the decrease in the spontaneous urge of the animal to move, an urge which is not affected by any other factor apart from the experiment. Use of mice as experimental animals allows the quantity of the substance under test to be kept at the minimum.

The method is as follows: The mouse is placed under a bell-jar on a circular sheet of paper coated with carbon black. The total number of paw prints arising, as the animal runs around, is recorded prior to and following treatment with the experimental substance and a comparison made.

Pharmacological investigations carried out on the milky juice of locally-cultivated *Lactuca virosa* (lactucarium) furnished renewed proof of its non-toxicity. In particular it was shown that *Lactuca virosa* has a pronounced sedative effect which can essentially be attributed to the two constituents lactucin and lactucopikrin.

Thus, observations of early physicians are understandable. They attributed to this medicinal herb a tranquillizing effect on the nervous system which they exploited practically by employing it as a means of combating cough.

When we began the investigations on the fresh latex, I was particularly impressed by the biochemical way of thinking of Willstaetter in Munich who examined the milky juice with respect to its enzyme content. By traditional methods, I experienced no difficulty in detecting a high content of peroxidase. The theory was quickly at hand, that the bitter principles which are easily oxidized by agents such as potassium peroxide are enzymatically decomposed by peroxidase. On the basis of this theory which subsequently proved to be partially incorrect, I developed a new method of extraction and drying which, in spite of the partially incorrect assumption, led to a stable product. To this extent, the theory seemed to be confirmed. The observations of Schmitt, who, just prior to the war, was working under my supervision for his doctorate, that lactucopikrin, on addition of methyl alcohol or hydrochloric acid to the fresh milky juice, remains stable whereas in the untreated milky juice it is quickly decomposed, supported our hypothesis that a fermentative process was involved in the decomposition of the bitter principles on drying.

Then came the war and our work was interrupted. My first scientific work in Berlin – I was given a professorship at the Free University in 1949 – was devoted to the question whether the theory of the destruction of the bitter principles based on the action of peroxidase was correct. After determination of the *p*H optimum of lactuca peroxidase, we carried out investigations on the damage caused to the enzyme on heating a mixture of the milky juice and water at various temperatures. My collaborator, Wendt found that at a temperature of 80° the enzyme was destroyed in a few minutes and that regeneration of the thus inactivated peroxidase after several days could not be found.

Experiments were now carried out with the aim of determining the temperatures at which the enzyme system responsible for the destruction of lactucopikrin could be eliminated. The following results were obtained. Although on heating at 80° for 10 min. the peroxidase loses its activity

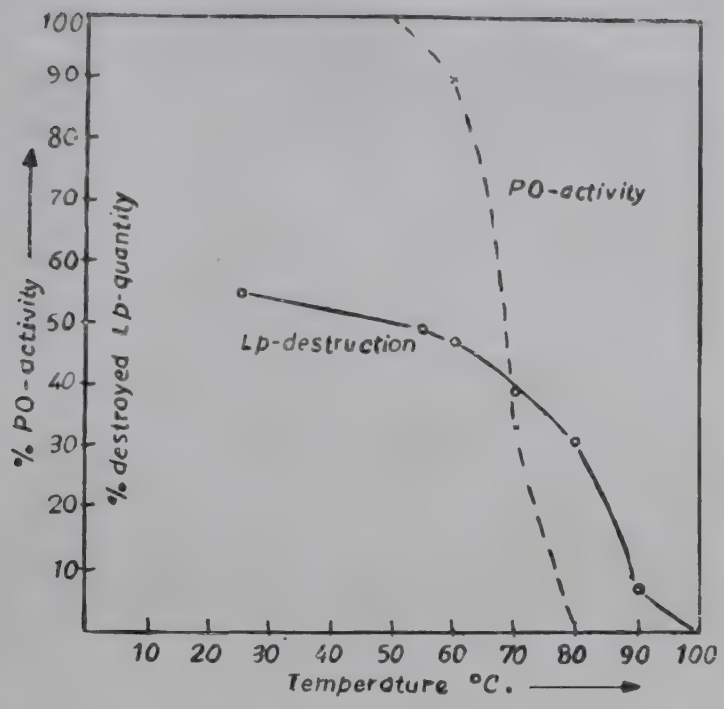


FIG. 1

completely, approximately 32 per cent lactucopikrin is, as a result of the same treatment, destroyed by another factor (Fig. 1).

From this we concluded that our theory of the fermentative destruction of the bitter principles in lactucarium and thus the destruction of the effectiveness, could only be partially correct.

Now I would like to deal with certain new investigations involving the effect of light on the stability of our preparations. On the occasion of the University Week of the Free University 1961, I gave a lecture in Mülheim on the Ruhr. After this lecture, I was invited to the house of my namesake Herr G. O. Schenck, Director of the Mülheim Max Planck Institute (Radiation Chemistry). During a long discussion lasting late into the small hours, he told me about his field of work, radiation chemistry and photochemistry.

Under the impression of this nocturnal conversation the thought went round in my mind, that the lactuca milky juice is dried in the sun on the banks of the Mosel. It immediately occurred to me that a photochemical process might be involved. The investigations which were carried out by Herr Naumann were completely successful. Lactucin is destroyed by light. Our working hypothesis that lactucin is decomposed by oxidation under the influence of peroxidase was thus incorrect and concerning lactucin partly correct.

In new experiments we found that lactucin is an extremely photosensitive substance.

The extent of photochemical conversion of lactucin in relation to the time of exposure to radiation can be followed spectrophotometrically

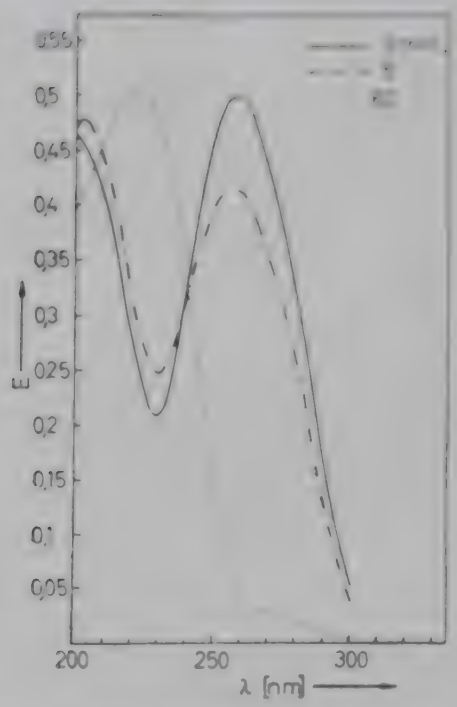


FIG. 2—CHANGING OF THE SPECTRA OF LACTUCIN DURING RADIATION

by the characteristic change in its absorption curve between 200 and 300 mμ.

As shown in Fig. 2 there is a continuous transition from the absorption curve of an aqueous solution of lactucin with a maximum at $\lambda = 259$ mμ to the common absorption curve of the two products with a new maximum at approx. 223 mμ. Under the above conditions the conversion process occurs within one hour. In direct sunlight the quantitative destruction takes place in 6 hr, in diffused daylight 6 to 8 weeks are required.

As a result of the photochemical conversion of lactucin in aqueous solution two new compounds are produced which can be identified by descending paper chromatography. Lactucin and its two decomposition products were identified as a result of fluorescence of their spots on the paper chromatogram in UV-light (254 mμ) or by spraying with an alcoholic solution of 2,3,5-triphenyltetrazolium chloride.

Comparison of the reaction rates in nitrogen and oxygen atmospheres under otherwise identical radiation conditions showed that oxygen played no part in the reaction. Lactucin in a buffered solution of pH 3 to 8 was completely stable when exposed solely to the action of oxygen.

The first photochemical product was obtained from the reaction mixture by repeated re-crystallization from ethyl acetate, pure white scaly crystals being produced. The R_f value in the above mentioned carrier amounts to 0·57 and thus differs noticeably from the R_f value of lactucin which, under the same conditions, amounts to 0·74.

From elementary analysis and determination of the molecular weight in a pressure osmometer a summation formula of $C_{15}H_{16}O_5$ was determined, which was thus identical with the summation formula of lactucin. In spite of the identical summation formulae, however, there exist differences in the UV and IR spectra of the two substances. We think that the photochemical reaction involves solely a process of isomerization of lactucin.

The R_f value of the second photochemical product amounts to 0·44 under the same conditions as were mentioned above for the first product. This compound was extracted column-chromatographically from the mother liquor remaining after isolation of the first secondary product. All attempts to recrystallize the substance resulted in an amorphous hygroscopic product.

From elementary analysis and determination of the molecular weight in a vapour pressure osmometer, a summation formula of $C_{15}H_{18}O_6$ was determined. From this it can be assumed that the compound arises as a result of photochemical addition of water to lactucin.

In the case of lactucopikrin, however, the less active component, we did find an enzymatic reaction, as it is an ester of lactucin and 4-hydroxyphenylacetic acid.

The behaviour of lactucopikrin in the presence of peroxidase and hydrogen peroxide was systematically examined. A reaction of the phenyl group with peroxidase is possible.

Even in the oxidation of 4-hydroxyphenylacetic acid with hydrogen peroxide and peroxidase in solutions buffered at *p*H 3·5 and 7, a change in the spectrum of the substrate was determined spectrophotometrically. The reaction proceeded most rapidly at *p*H 5. For complete oxidation, two oxidation equivalents of hydrogen peroxide were required per mole of phenol. The results of spectrophotometric investigations were confirmed by paper and thin layer chromatography. The chromatographic properties of the oxidation products arising, suggested the existence of polymeric substances. After oxidation of 4-hydroxyphenylacetic acid with 1 oxidation equivalent of hydrogen peroxide, a small yield of a trimeric substance was isolated.

On oxidation of the ester lactucopikrin with hydrogen peroxide and peroxidase, oxidation products were obtained which were insoluble in water. It was subsequently found by quantitative determination with methanolic potassium hydroxide that the lactucin component remained unaffected during the process of oxidation.

The result of our investigations on the latex of *Lactuca virosa* was that we found an enzymatic effect on lactucopikrin and an effect of light on lactucin and lactucopikrin.

Neuropharmacological Profile of Jatamansone with Special Reference to its Effectiveness in Hyperkinetic States

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New Delhi, India

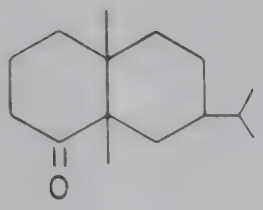
Introduction

Nardostachys jatamansi has been used since 1000–800 B.C. in the treatment of a variety of mental disorders such as epilepsy, hysteria, chorea and other nervous disorders. Charak, Sushruta and Vagbhatta, the three earliest and most eminent scholars of ancient Indian medicine, used *jatamansi* for treatment of mental disorders. Sushruta gives a very good description of insanity, in which, states of over-talking, laughing, singing and crying, aimless roaming, overeating, excitement, peculiar dressing and quarrelsomeness are described. Sushruta administered *jatamansi* to cause sedation in all these mental disorders and particularly in those where the signs and symptoms of the disease are the same as are found in hyperkinetic patients.

Nardostachys jatamansi (Unani name: *sumbul lateeb* or *balchar*) has also been used in the Unani or Muslim system of medicine since the time of Avicenna (Sheikh Bu Ali Seena, A.D. 980–1033), who was the author of 'Cannon of Medicine'. *N. jatamansi* is described as a hypotensive drug and useful in palpitation of the heart. It is also recommended for weakness of the brain and in epilepsy. Al Qanoon (Vol. 2, pp. 133-34, lines 25–30) contains most informative reference to *jatamansi*, here called *bal-har*.

Chemistry of Jatamansone

Jatamansone is a sesquiterpene ketone present in the rhizomes of *jatamansi*[1] and in the roots of *Valeriana officinalis*[2,3]. Detailed structural



I

FIG. 1—STRUCTURE OF JATAMANSONE

investigations on this compound were conducted simultaneously and independently by Govindachari and coworkers[4–6], and by Sorm and co-workers[3,7–9]. The latest investigations by the latter group have shown that jatamansone is correctly represented by (I) (Fig. 1).

Hypotensive Action

Studies on jatamansone in different species conducted in our laboratory are presented in Table 1.

Clinical trials to examine the antihypertensive action of jatamansone were conducted on 22 patients at Safdarjung Hospital, New Delhi, under the guidance of M. S. Rao and on 6 patients at K. E. M. Hospital, Bombay, under the guidance of U. K. Sheth and L. P. Shaw. Antihypertensive agents, viz. reserpine, syrosingopine, guanethedine, bretylium tosylate and ganglionic and adrenergic-blocking agents, which act upon the sympathetic nervous system, exhibit characteristic effects on the nictating membrane of cats and upon certain autonomic and cardiovascular responses in the dog.

With this in mind, experiments were conducted to determine if jatamansone also possessed any properties in common with such agents. Central site of action does not seem to play an important role in the mechanism of hypotensive activity of this drug since, when jatamansone was injected directly into the lateral cerebral verticle, hypotensive action was not significant.

Biochemical approach to mechanism of action of jatamansone

Reduction in the level of brain norepinephrine, started 4 hr after jatamansone injection, reached the maximum at 24 hr and had not touched normal after 48 hr, the period up to which the study was made. This is shown in Fig. 2.

Assays of 5-hydroxytryptophan decarboxylase and monoamineoxidase were carried out 8 hr after feeding 100 mg./kg. body weight of jatamansone and of 5-hydroxyindoleacetic acid on urine after 24 hr. These results are shown in Table 2.

Earlier, Arora *et al.* [10,11] had shown that jatamansone possesses mild tranquillizing effect on unanaesthetized mice and antiepileptic activity in various animal species. It reduced spontaneous motor activity, and

TABLE 1 –HYPOTENSIVE EFFECT OF JATAMANSONE ON ANIMALS

Animal species	Dose *mg./kg.*	Percentage fall	Number of experiments	Onset of action following injection	Period of max. action following injection	Remarks
Cats[1]	20 to 25 mg. i.v.	50-60	150*	Immediately	1½ to 3 hr	Response noted in 90% of animals
Dogs[2]	25 to 30 mg./i.v.	20-25	20	do	do	Marked variation in response from animal to animal
Monkeys[3]	20 to 25 mg./i.v.	40-50	6	do	do	Animal variation in response was less compared to dogs
Rabbits[4]	20 to 25 mg./i.v.	30-40	6	do	do	do
Pigs[5]	25 to 30 mg.	20-25	6	do	do	Marked variation in response from animal to animal
Rats[6]	50 mg./kg. i.p. & 100	20-28	20	4 hr	8-12 hr	Positive response noted in 90% of animals
Rats[7]	10 & 30 mg./kg. i.p.	25-30	20	1 hr	3 and 5 hr	do
Frogs[8]	25-30 mg. kg. i.v.	40-50	22	3-5 min.	0.5 to 1 hr	Positive response noted in 80% animals

*Mechanism of hypotensive action of jatamansone was studied on cats. Hence the number of experiments on cats is more than in any other species.

1,2,3,4,5 : Hypotensive action determined on anaesthetized normotensive animals.

6 and 7 : Hypotensive action determined on unanaesthetized normotensive and hypertensive rats respectively.

8 : Hypotensive action determined on anaesthetized normotensive frogs.

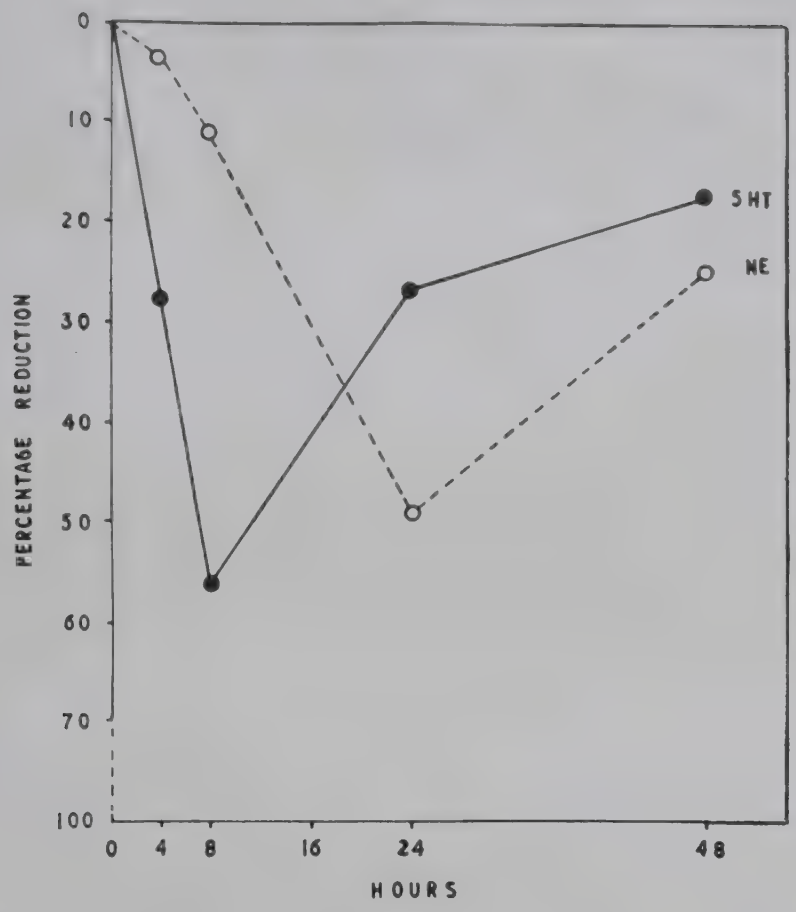


FIG. 2 — BRAIN NEURO-EPINEPHRINE LEVEL FOLLOWING INJECTION OF JATAMANSONE

TABLE 2—5-HYDROXYTRYPTOPHAN DECARBOXYLASE MONOAMINE-OXIDASE AND 5-HYDROXYINDOLEACETIC ACID

5-HYDROXYTRYPTOPHAN DECARBOXYLASE			MONOAMINEOXIDASE			5-HYDROXYINDOLE-ACETIC ACID		
Normals (10)	Treated (10)	% Inhibition	Normals (10)	Treated (10)	% Inhibition	Normals (10)	Treated (10)	% Decrease
μl. CO_2 evolved/g. Tissue/hr			μlO_2 uptake/g. Tissue/hr			mg./24 hr Urine		
79·7 ±2·05	57·8 ±2·71	27	150·6 ±1·85	151·9 ±1·89	Nil	0·49 ±0·06	0·17 ±0·03	66

Figures in parentheses denote the number of animals in each group

NOTE: 5-Hydroxytryptophan decarboxylase and monoamineoxidase assays were carried out after 8 hr of 100 mg./kg. body weight jatamansone administration, while 5-hydroxy-indoleacetic acid estimation was on 24 hr urine sample.

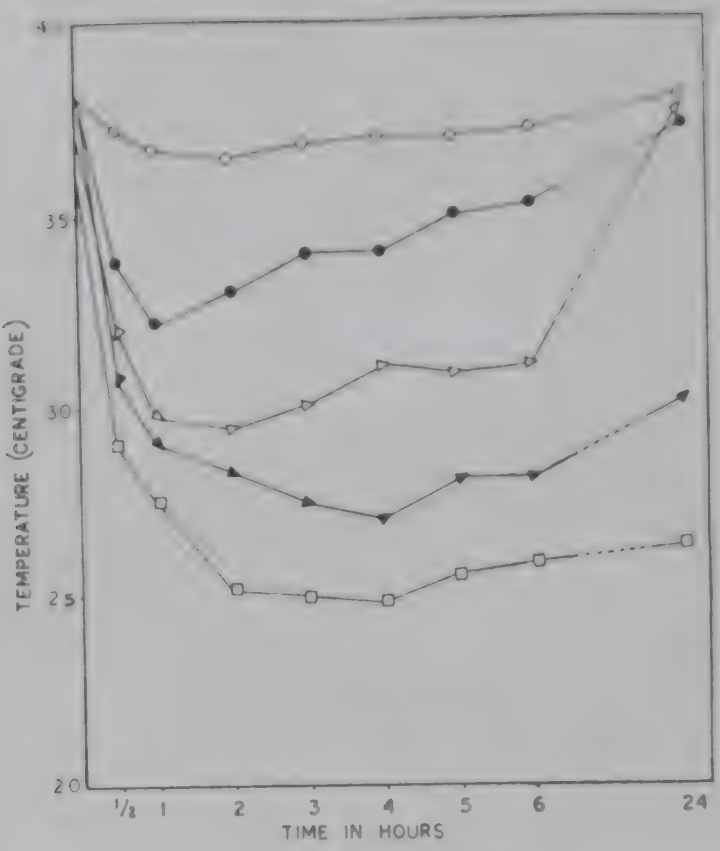


FIG. 3—HYPERTHERMIC EFFECT OF JATAMANSONE IN MICE

100 mg. kg. of jatamansone reduced the emesis provoked by apomorphine hydrochloride by an average of 70 per cent in the mongrel dog. It also produced hypothermia in mice (Fig. 3).

Jatamansone, administered orally as well as intraperitoneally, potentiated the effect of pentobarbitone sodium. In the oral route, the potentiating effect was constant at doses from 10 mg. kg. onward, and with intraperitoneal administration the potentiating effect was constant and significant from a dose of 1 mg. kg. onward. There was no significant difference in means between the sleeping time at two different environmental temperatures of 28° and 38°C. These results are shown in Fig. 4.

To really study the effect of a drug on the central nervous system, a battery of different experiments has to be used instead of one or two indicators of central activity. Since knowledge of the seat of emotions in the brain is still inadequate, the effect of a new drug has to be based on indirect experiments.

Information obtained from the literature and from our studies indicated that the drug might possess interesting neuropharmacological properties. To examine these, a number of studies were carried out.

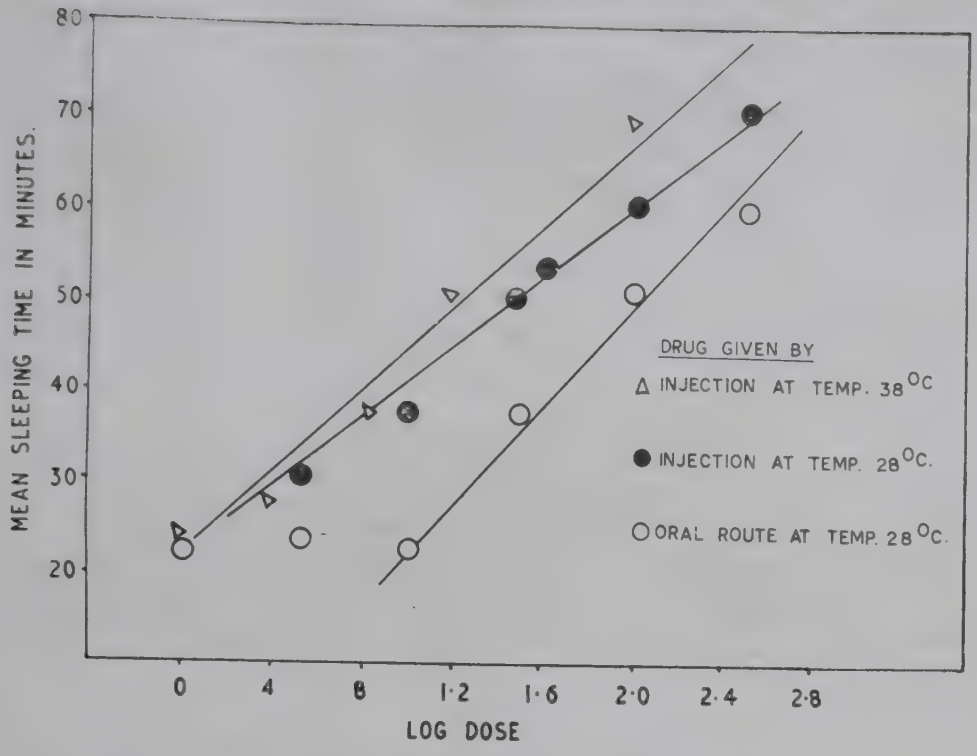


Fig. 4

EXPERIMENTAL

Neuropharmacological Studies on Jatamansone

Behavioral effects of jatamansone. Effects of jatamansone on the behaviour of unanaesthetized monkeys were examined. The behavioral pattern was scored following Norton[12]; the scoring sheet is given below:

Sociability: Grunt, can be touched, tugs at observer, takes object, comes forward.

Contentment: Play (chewing paper, cage), hooting, grooming, eating, resting (sitting).

Excitement: Chatter, mouthing, jumping around, rattles chain, comes forward.

Hostility (Defensive): Withdrawing, urination, baring teeth, pulling back, open mouth.

Hostility (Aggressive): Anger bark, head lowering, crouching, leaning forward, swatting (grabbing).

Chlorpromazine and reserpine, the two major tranquillizers, and other psychotropic drugs produce changes in the behavioral pattern. With chlorpromazine, sociability is increased, contentment is unchanged, defensive hostility is decreased and slight increase is found in aggressive hostility[12]. Jatamansone reduced excitement, and defensive and aggressive hostility, while sociability and contentment were increased. The results are shown in Fig. 5.

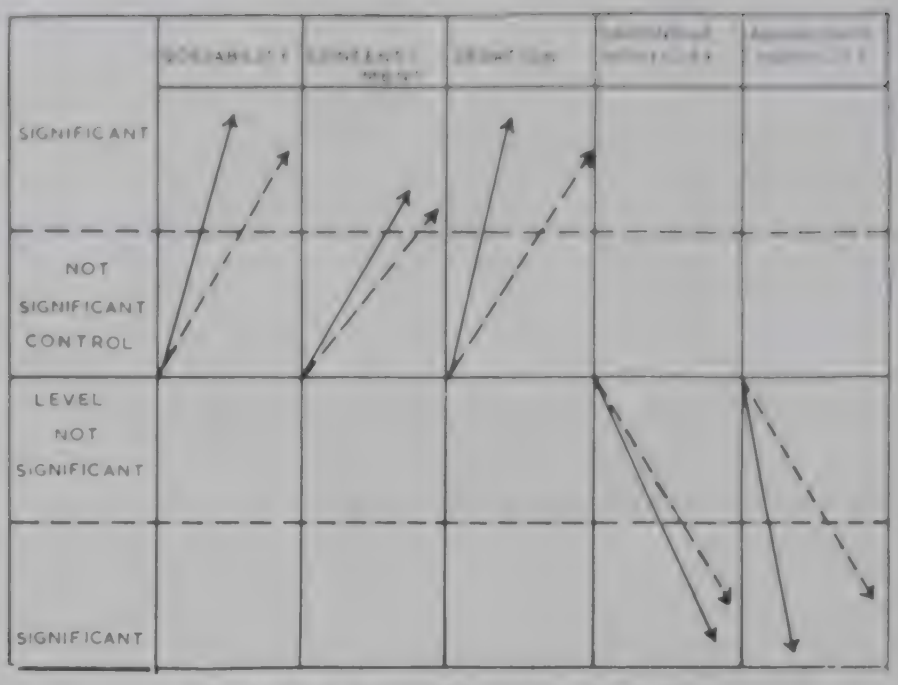


FIG. 5—BEHAVIORAL EFFECTS OF JATAMANSONE ON UNANAESTHETIZED MONKEYS

Conditioned avoidance response in rats. Inhibition of the conditioned response was used as a measure of tranquillization, and the percentage of failure in five trials as a measure of potency. At a dose of 100 mg./kg. there was no significant effect. At 300 mg./kg., conditioned response was inhibited; the effect started 3 hr after drug injection and was maximum between 6 and 8 hr. The effect lasted up to 12 hr. These findings are depicted in Fig. 6.

Morphine rage in cats. Morphine produces a typical maniacal state in cats characterized by restlessness, dashing against the cage wall, maniacal bouts and mydriasis. The effect of tranquillizing drugs on such morphine reaction have been studied in this species. Chlorpromazine and reserpine both lessen the severity of morphine excitement. The mydriatic effect of morphine predominates over the miotic effect of the tranquillizer[18]. Reserpine is reported to counteract the manifestation of slow rage in cats. Bearing in mind the results with chlorpromazine and reserpine, the effect of jatamansone on morphine mania in cats was examined. Morphine rage, characterized by excitement, apprehension, ataxia and marked mydriasis was first produced in cat. When 20 mg./kg. morphine was administered subcutaneously, the rage phenomenon appeared within 45 min. of injection of morphine sulphate and lasted for 6 hr. Two cats were kept as control receiving only the morphine injection. Four cats each were treated intraperitoneally with jatamansone at a dose of 100 mg./kg. and 300 mg./kg., followed, 3 hr later by subcutaneous injection of 20 mg./kg. of morphine sulphate. The onset, severity and duration of rage phenomenon

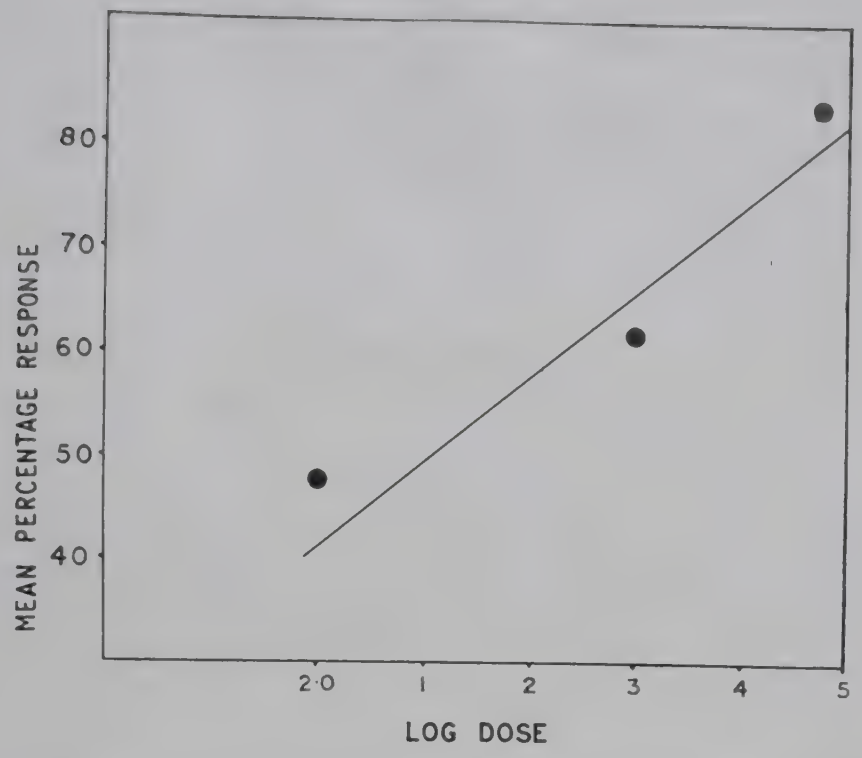


FIG. 6—EFFECT OF JATAMANSONE ON CONDITIONED AVOIDANCE RESPONSE IN RATS

in the cats given jatamansone were compared with the control cats. Jatamansone given in the smaller dose of 100 mg./kg. delayed the onset and reduced the intensity of mania. The 300 mg./kg. dose abolished the rage completely, but mydriasis still persisted.

Amphetamine toxicity in mice. In 1940, Gunn and Gurd[14] reported that mice which were grouped with other mice were more susceptible to the excitatory effects of amphetamine and allied drugs than the single mouse. Chance[15] reported experiments investigating the relationship of confinement and aggregation to amphetamine toxicity. Mice that were crowded together were reported to die from much smaller dose of sympathomimetic amines than did mice in a less crowded environment. With amphetamine, the LD_{50} changed to ten-fold as a result of the population density[15]. In mice injected with amphetamine and placed crowded in a box, the mortality was due to excitement and fright and not to amphetamine. Tranquillization is essentially an action in which excitement is suppressed, and this provides a means of testing tranquillizing drugs. Burn and Hobbs[16] reported that the LD_{50} of amphetamine is about 100 mg./kg. if mice are separate from one another, but is about 14 mg./kg. if they are placed ten in a box. When together, they excite one another and die from excitement. Chlorpromazine and reserpine both provided protection from amphetamine toxicity[17]. Chlorpromazine abolishes the excitement when given one hour previously in doses as small as 0.3 mg./kg., which corresponds to 20 mg. for a man of weight 70 kg. The effects of reserpine are similar in doses of

and mg./kg. or less when given 4 hr before amphetamine. The method is completely objective as the results are expressed in terms of the number of mice killed.

In the present work, similar studies were conducted with jatamansone. Male albino mice weighing 20 to 24 g. were used. They were not deprived of food before the experiment, but during the test they were not given food. Mice were kept, 10 together, in wire-mesh cages 27 cm. long, 17 cm. wide and 15 cm. deep. The cages were kept in a small room at a constant temperature of 28°C.

Jatamansone at levels of 30 and 100 mg./kg. was injected after amphetamine sulphate. The mice were then observed twice during the day. The final count of dead mice was done the following morning. The results are depicted in Table 3. Jatamansone did not protect the aggregated mice against amphetamine toxicity. There was slight increase in toxicity. Control mice receiving Tween 80 also showed slight increase in toxicity of amphetamine.

Leptazol convulsions. Reserpine has been shown to shorten the survival time of mice infused intravenously with leptazol[18]. Keeping in mind the different results obtained with reserpine, chlorpromazine and barbiturates on leptazol convulsion, we thought it worthwhile to study the effect of jatamansone on leptazol convulsions in rats.

Adult albino rats, in groups of 10 each, received 30 and 100 mg./kg. of jatamansone intraperitoneally, followed, 3 hr later, by subcutaneous injection of 70 mg./kg. leptazol. One group of animals received only leptazol (70 mg./kg.) subcutaneously and served as control. Rats were kept in a small room at a constant temperature of 28°C. and observed two to three times a day, the final count of dead rats being made at the end of 24 hr.

Jatamansone did not protect the rats from leptazol toxicity. On the other hand, there was some increase in death rate. Findings are depicted in Table 4.

Deaths occurred during severe tonic convulsion phase within 4 hr of injecting leptazol. Increase in leptazol toxicity could be due to hypothermia produced by the drug, as it has been observed that lowering of body temperature increases the tendency of central nervous system to respond to analeptics with convulsions and tetanic discharges.

TABLE 3. EFFECT OF JATAMANSONE ON AMPHETAMINE TOXICITY IN MICE: SOLVENT CONTROL EMULSION OF 'TWEEN 80' IN WATER

Drug	Dose mg./kg.	Amphetamine sulphate 14 mg./kg. subcutaneous	Mortality	
			1st Expt	2nd Expt
Jatamansone	30	Injected	7/10	8/10
Jatamansone	100	Injected	10/10	9/10
Solvent	100	Injected	9/10	9/10

TABLE 4—EFFECT OF JATAMANSONE ON LEPTAZOL CONVULSIONS

Drug	Dose *mg./kg.*	Leptazol 70 mg./kg. subcutaneous	No. of rats	Percentage mortality
Control		Injected	10	60
Jatamansone	30	Injected	10	70
Jatamansone	100	Injected	10	70
Solvent	100	Injected	10	60

Toxicologic evaluation of jatamansone. Prolonged toxicity studies with jatamansone were conducted to find out the safe therapeutic dose and also to reveal the toxic effects which might arise after repeated administration of the drug. The drug was administered to rats every day for 52 weeks. The animals were closely observed throughout the experiments for signs of toxicity like loss of weight, anorexia, ataxia, paralysis, tremors, convulsions, muscular weakness, pupillary changes, salivation, vomiting and diarrhoea. Results showed that apart from sedation, there was no other observable functional change in the animal, at daily doses of 30 mg./kg. of the drug which is 20 times the effective human dose. Mild chronic toxicity, shown in Fig. 7, was observed in the form of slight fatty and cloudy degeneration in liver and kidney at 100 mg./kg. daily dosages in rats.

Teratogenic effect in animals. These studies were done on pregnant rats. The rapidly-growing rat embryo has simultaneous proliferation of all systems at accelerating or decelerating rates as different developmental stages progress. The rat embryo has an average gestation period of 22 days, and organogenesis proceeds rapidly after the seventh day. The end of metamorphosis occurs at about 16 days, the bones begin to ossify on the 16th day. The drug at a dose of 50 mg./kg. has no effect on the number of litters. In other words, unlike reserpine, it has no antifertility effect. There was no case of abortion and no significant change in the gestation period. No abnormality was observed either in size or in the external appearance of the new-born pups. The growth of the pups was normal.

Preliminary Clinical Trial of Jatamansone on Hyperkinetic Patients

The drug was clinically tried on hyperkinetic patients in the Psychiatry Out-patients Department of the Willingdon Hospital and Nursing Home, New Delhi, with the collaboration of A. K. Chatterjee. Details of the work done and the results obtained follow.

Procedure followed for double-blind clinical trial of jatamansone. Four cases were selected for trial. All the four cases were male children between 8 and 12 years of age. All of them were cases of mental deficiency with behavioral disorder, hyperkinesis, restlessness, and aggressive and destructive tendencies. Symptoms of the patients were divided into various

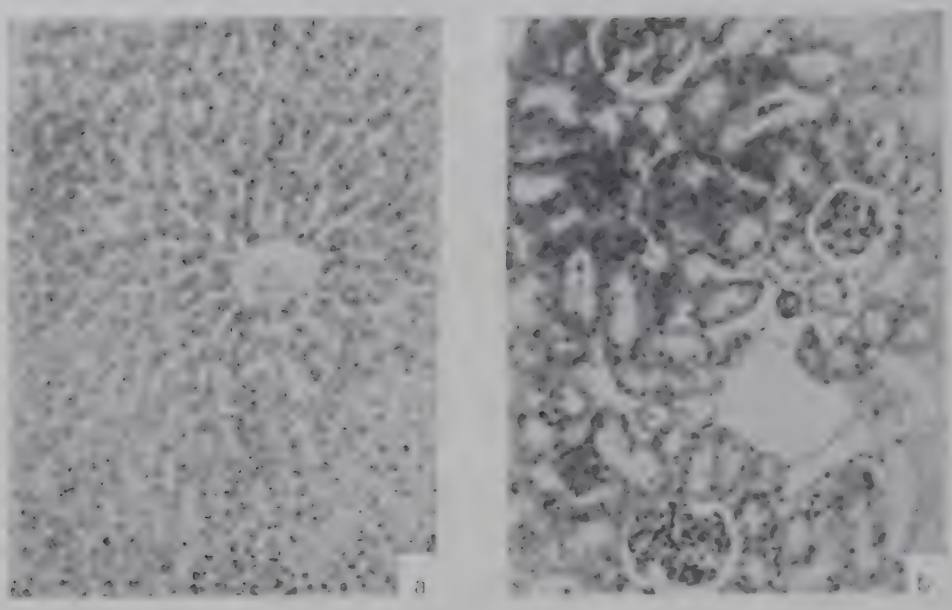


FIG. 7

classes such as aggressiveness, destructive tendencies, shouting, crying, abusing, insomnia, restlessness and stubborness.

The patients had not received any treatment for hyperkinesis for a period of 8 months before starting the trial. Before this period, several tranquillizers such as chlorpromazine trifluprornazine, trifluperazine and melliril (thioridazine), had been tried on them with no beneficial effect.

Before the trial, the patients and their parents were interviewed and symptoms of the patients assessed. The investigations done before the trial with the drug were: (i) examination of blood (haemoglobin, total and differential W. B. C.s count, total R. B. C.s count), (ii) urine, (iii) stool and, (iv) electrocardiogram.

These tests were repeated every 15 days during the trial. Before starting the trial, the blood pressure of the patients was recorded; during the trial, it was recorded on each visit of the patient, i.e. three times a week. This was thought important because, as already reported by us, jatamansone possesses antihypertensive activity.

An emulsion of jatamansone was dropped into capsules containing glucose A. R. and the capsules were sealed. Identical capsules were prepared for placebo. Initially 20 mg. day of jatamansone was given. The dose was increased up to 60 mg. day during the period of treatment with the drug (3 to 5 weeks). This followed treatment with placebo for 3 to 5 weeks.

As mentioned earlier, the patients and their parents were interviewed and symptoms of the patients assessed. Beneficial effects on patients in their response to the drug were categorized as follows: Very good — two patients; Good — one patient and, Doubtful — one patient.

It is evident from the findings that in all four cases there was initial response in the form of reduced aggressiveness, destructiveness, restlessness

and stubborness. All the patients complained of insomnia before the trial which was completely cured after administration of the drug.

However, the response seems to wane in a few days' time and increasing doses were required to maintain the therapeutic response. The effective dose for a beneficial effect ranges from 20 to 60 mg./day.

During and after the 3–5 weeks trial no toxic effects were detected on clinical examination and on examination of blood, urine and stool. No change was found in the electrocardiogram. No appreciable fall in blood pressure was noted in the patients, all of whom had a normal blood pressure before the trial. Seven to 10 days after starting placebo, the condition of the patients began deteriorating. At the end of 3–5 weeks treatment with placebo, their condition became almost the same as it was at the beginning of the trial.

The drug is effective in hyperkinetic patients and is free from troublesome side effects. Development of tolerance poses the problem of maintenance of an effective dose, but the desired response can be maintained if the doses are increased frequently. Clinical trials using more cases are in progress.

Experimentally-induced Hyperactivity in Animals

Literally speaking hyperkinesia means abnormally increased mobility or abnormally increased motor function. An attempt was made to create hyperkinetic states of hyperactive condition in animals by means of surgical ablation and drugs, which more or less simulated the hyperkinetic state in children. As a result of recent study by various workers[19], frontal lobe of brain has emerged to be primarily concerned with elaboration of effective behaviour. Various types of changes in the effective behaviour have been reported by many workers after the involvement of posterior orbital cortex and head of the caudate nucleus in frontal lobotomies[20–23].

MATERIALS AND METHODS

(a) *Animals*: Hyperactive state in monkeys was produced by bilateral surgical ablations involving frontal lobe structures. After studying the effect of these operations on behaviour, beneficial effects of jatamansone were studied.

Monkeys weighing 3–5 kg., who looked quite healthy, were kept at room temperature 75°–80°F. Diet consisting of groundnuts, gram, oiled bread, bananas and water was given. The animals were allowed to get acclimatized to the laboratory conditions for a period of about 1–2 weeks. The behaviour of the animals was then studied for 3–4 weeks before subjecting them to any operative procedure. Subsequently, behavioral changes were studied after operation.

(b) *Operative procedure*: Frontal lobe surgical lesion was produced in one stage. Animals were anaesthetized by intraperitoneal injection of pentobarbitone sodium in a 5 per cent solution. The dosage used for monkeys was 0·7 ml. of 5 per cent solution per kg. body wt. The animals usually

became completely anaesthetized in about 15 to 25 min. An incision parallel to the superior orbital margin was made according to the requirements. Skin and occipito frontalis were cut, the periosteum elevated and bone exposed. Holes were drilled over the required area and widened to have a good view of the duramater. Blood vessels in the duramater were cauterized. It was cut in cruciate fashion and the frontal lobe of the brain was exposed. The part to be removed was identified and the lesion was made by aspiration. To the naked eye, these lesions appeared to involve the posterior orbital cortex and head of the caudate nucleus of the frontal lobe of the brain. The bleeding was arrested by warm saline packing. Packs were removed and dura was closed. Occipito frontalis muscle and skin were sutured, and a thin cotton pad soaked in tincture benzoin compound was applied over the sutured wound and allowed to dry. Procaine penicillin (0·4 million units) were given intramuscularly for 5 days to avoid risk of infection.

These monkeys became hyperactive about 10–23 days after the operation. During the day, they continuously paced about the cage, from 20–40 times per minute. The hyperactivity increased whenever a test was conducted. Most of these monkeys were fearless but not aggressive. Only one monkey developed aggressive and hostile behaviour. The behaviour was observed for 6 weeks before jatamansone was administered.

Effect of Jatamansone

Stock solution of jatamansone was made in 5 per cent Tween 80. Control studies with 5 per cent Tween 80 revealed that this solvent possesses no effect on behaviour of the monkeys. Jatamansone in doses of 40, 50, 60, 70 mg./kg. was injected intraperitoneally into a group of monkeys. One monkey served as control. The onset of action of jatamansone was 1 hr, 40 min. and 30 min. respectively. The duration of action was for about 22 hr, 28 hr, 34 hr and 40 hr. The reduction of hyperactivity, based on hypermotility was 30–40, 50–60, 70–80 and 85–100 per cent respectively. No toxic manifestation could be seen during the trial of the drug. These monkeys fully recovered their hyperactivity with the stoppage of the drug.

In another group of monkeys 100 mg./kg. of jatamansone was injected intramuscularly. The onset of action was quite delayed, about 3–5 hr after administration, the hyperactivity being reduced by 45–55 per cent. These results are shown in Table 5.

In a group of monkeys 55 mg./kg. of jatamansone was given intraperitoneally daily for a few days. After about 4 hr of injection on the 2nd day, the hyperactivity of monkeys was reduced by 95–100 per cent and this reduction in hyperactivity could be maintained by a daily injection of 55 mg./kg. i.p. Since only limited quantities of the drug were available, this experiment could not be conducted for a long period, and further work is required to reach a definite conclusion.

TABLE 5—EFFECT OF JATAMANSONE ON HYPERACTIVE MONKEYS (MEAN AVERAGE FINDINGS)

(Control: Emulsion of 5% Tween 80 in water)

Drug & Dose *mg./kg.*	Route of administration	Onset of action *hr min.*	Duration of action *hr*	Reduction of hyperactivity %
Jatamansone 40	i.p.	1·00	22	30 to 40
Jatamansone 50	i.p.	1·00	28	50 to 60
Jatamansone 60	i.p.	0·40	34	70 to 80
Jatamansone 70	i.p.	0·30	40	85 to 100
Jatamansone 100	i.m.	3-5·00	45-50	45 to 55

The beneficial effect of jatamansone on hyperactive monkeys was compared with that of reserpine and chlorpromazine. Reserpine (0·06 mg./kg.) given intramuscularly did not have any beneficial effect. Chlorpromazine (0·4 mg./kg.) given intramuscularly reduced the hyperactivity by 10–20 per cent only.

Drug-induced Hyperactivity in Animals

These studies were conducted in mice with amphetamine sulphate (Dexedrine). Hyperactivity was created in mice by injecting 10 mg./kg. amphetamine sulphate subcutaneously[24]. After about 20 min. these mice became aggressive, hostile and hypermotile, characterized by fast and continuous multidirectional movements. This effect lasted for more than 5 hr. Male mice weighing between 20 and 25 g. were divided into 4 groups of 8 animals each. One group of mice was kept as control and given only distilled water subcutaneously. Another group was given amphetamine sulphate (10 mg./kg.). A third group was given amphetamine sulphate (10 mg./kg., subcutaneous) and after 30 min. jatamansone (60 mg./kg.) was injected intraperitoneally. In the fourth group, jatamansone (60 mg./kg.) was injected intraperitoneally and after one hour amphetamine sulphate (10 mg./kg., subcutaneous) was injected.

There was no behavioral change in the control group; characteristic hyperactivity was exhibited in the second group. In the third group, aggressiveness and hostility of mice were abolished completely, with 25–40 per cent reduction of hypermotility, while in the fourth group the onset of action of amphetamine sulphate was delayed 40 min. after the injection and at the same time aggressiveness and hostility completely disappeared and hypermotility was reduced by 25 to 40 per cent.

CONCLUSION

Jatamansone is a sesquiterpene ketone isolated from the roots and rhizomes of an Indian indigenous plant *Nardostachys jatamansi*. This drug in its crude form has been used since time immemorial for various types of

nervine disorders, e.g. epilepsy, hysteria and convulsive disturbances. Barbey[10,11] Jatamansone was shown to possess tranquillizing and antiepileptic activity in various animal species. It reduces spontaneous motor activity, produces hypothermia, increases pentobarbitone Na-sleeping time, inhibits conditioned avoidance response in rats and suppresses morphine rage in cats. Information obtained from literature and from our studies indicates that this drug might well possess an interesting neuropharmacological profile. Subsequent to demonstration of uneventful acute, subacute and chronic toxicity, the drug was put to clinical trial in patients with hyperkinetic disease and the results have been promising. Bilateral frontal lobotomy produces hyperactive state in monkeys, which simulates the hyperkinetic states seen in children. Jatamansone is very effective in reducing the hyperactivity in hyperkinetic children and experimentally-induced hyperactivity in animals. Reserpine does not have any beneficial effect in these cases while chlorpromazine is little effective.

REFERENCES

1. Govindachari, T. R., Rajadurai, S. & Pai, B. R., *Chem. Ber.*, **91** (1958), 908.
2. Stoll, A., Seebeck, E. & Stauffacher, D., *Helv. Chim. Acta.*, **40** (1957), 1205.
3. Krepinsky, J., Romanouk, M., Herout, V. & Sorm, F. *Chemicke Listy*, **52** (1958), 1564.
4. Govindachari, T. R., Pai, B. R. & Rajadurai, S., *Tetrahedron Letters*, No. 15 (1959), 5.
5. Govindachari, T. R., Pai, B. R., Purushothaman, K. K. & Rajadurai, S., *Chem. Ber.*, (1960), 1059.
6. Djerassi, C., Govindachari, T. R., Pai, B. R. & Purushothaman, K. K., *Tetrahedron Letters*, No. 6 (1961), 226.
7. Krepinsky, J., Herout, V. & Sorm, F., *Tetrahedron Letters*, No. 7 (1960a), 9.
8. Krepinsky, J., Romanouk, M., Herout, V. & Sorm, F., *Tetrahedron Letters*, No. 7 (1960b), 9.
9. Krepinsky, J., Romanouk, M., Herout, V. & Sorm, F., *Tetrahedron Letters*, No. 5 (1962), 169.
10. Arora, R. B., Singh, M. & Chander Kanta, *Life Sci.*, **6** (1962), 225.
11. Arora, R. B. & Arora, C. K., Pharmacology of Oriental Plants, Proceedings of the Second International Meet., Czechoslovakia, Vol. 7, (1963), p. 51, Pergamon Press.
12. Norton, S., Psychotropic Drugs, (ed. Carattini, S. and Ghetti, V.), (1957), 76. Elsevier, Amsterdam.
13. Sturtevant, F. M. & Drill, V. A., *Nature* (Lond.), **179** (1957), 1253.
14. Gunn, J. A. & Gurd, M. F., *Physiol.* (Lond.), **97** (1940), 453.
15. Chance, M. R. A., *J. Pharmac. exp. Ther.*, **87** (1956), 214.
16. Burn & Hobbs, *Arch. int. Pharmacodyn.*, **113** (1957), 290.
17. Lasagna, L. & Mc. Cann., W. P., *Science*, **125** (1957), 1241.
18. Chen, G., Ensor, C. R. & Bohner, B., *Proc. Soc. exp. Biol. (N.Y.)*, **86** (1954), 397.
19. Fulton, J. F., *Yale Rec. Med.*, **26** (1953), 497; Govindachari, T. R., *Chem. Rev.* (1960), 908.
20. Kluver, Hand Bucy, P. C., *Archs. Neurol. Psychiat. Chicago*, **42** (1939), 979.
21. Bard, P. & Mountcastle, V. B., *Res. Publ. Ass. Res. nerv. ment. Dis.*, **27** (1948), 362.
22. Maclean, P. D. & Clin, E. E. G., *Neurophysiol.*, **4** (1952), 407.
23. Anand, B. K., Duca, S. & Chhina, G. S., *Indian med. Res.*, **45** (1957), 353.
24. Maffii, G. & Soncin, E., *J. Pharm. Pharmac.*, **10** (1958), 541.

Further Experiences with a Stable Fraction of *Paspalum scrobiculatum* (Harik) in the Management of Excited Psychotics

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In the region around Bombay, a variety of millet grain, *Paspalum scrobiculatum*, known locally as Harik or Kodro, is used as a substitute for rice by poor people. At times a particular variety of this millet produces toxic reactions both in animals and human beings. This toxic variety of millet has also been called 'mino-kodro'— the word 'mino' indicating drowsiness. Such toxic variety occurs especially in the year when rains are excessive. Farmers carefully separate the husk from the grains, as the consumption of both by the cattle produces toxic actions. These toxic actions are manifested by drowsiness, vomiting, tremors and giddiness.

Historically speaking, these toxic symptoms have been described by Chanakya (321 B.C.). This was also described by Narhar (A.D. 1400), Ramdas (1636), Chevers (1870), Swarup (1922) and Sundaram Ayyar and Narayanswami (1946).

The CNS depressing effect in experimental animals with dried ethanol extract prepared from the husk of the grain of *Paspalum scrobiculatum* was first reported as a preliminary communication by Bhide and Aiman (1959), and subsequently in greater detail by Bhide (1962). The extract produced CNS depression and tremors in various species of animals, potentiated the effect of hexobarbitone in mice, produced hypothermia in rats and a decrease

in morphine-induced rage in cats. Acute and sub-acute toxicity studies in animals suggested a considerable margin of safety (Bhide, 1962).

Preliminary clinical trials by Dev and Bhide (1963) indicated tranquillizing effect of the extract in acutely disturbed schizophrenic patients. Dev (1964) reported further studies with same fraction in a double blind cross-over study of 40 excited psychotic patients. In the same year, we reported identical results in 25 excited patients treated with the same fraction (Shah *et al.*, 1964). Our further experience with a stable fraction of *Paspalum scrobiculatum* (prepared by Dehwala) in the management of 19 excited psychotic patients, is reported here.

Method of Preparing Active Fraction

Seeds and husk of toxic varieties of Harik (*Paspalum scrobiculatum*) were extracted with ethanol as described by Bhide. The extract was concentrated under vacuum at low temperature to obtain a dark viscous residue. This residue was titrated with ether several times and the pooled ether extracts after dehydration with anhydrous sodium sulphate was distilled under vacuum to get a grey coloured semi-solid. Further purification of the semi-solid by means of column chromatography resulted in obtaining three fractions of which, the fraction H-3, a buff-coloured solid was found to be biologically highly active, whereas the other fractions, including a white crystalline solid (m.p. 80–81°C.) were devoid of biological activity. H-3 itself was shown to be a mixture of closely related substances. Further work on the separation of H-3 into its components is in progress. The fraction H-3 does not contain any nitrogen or sulphur. It has been found to be stable to heat and storage. No appreciable loss in its biological activity was observed even after a long period of storage for over 8 months at room temperature or by boiling in organic solvents for 6–8 hr.

Selection of Patients

Patients for this trial were selected from Out-patient Section of the Psychiatry Department of K.E.M. Hospital, Bombay. Patients with varying grades of increased psychomotor activity manifested by outbursts of aggression and violence, rowdyism, destructiveness and assaultive behaviour were hospitalized and graded on a five-point scale before treatment.

Twenty-one male patients of different ages, between 18 and 55 years, were included in this study. Of these patients, two inadvertentiy received a less potent fraction and are not included in final assessment. The degree of excitement and diagnosis is shown in Table 1.

This was agreed upon by more than two independent observers. The duration of the excitement in these patients varied between 2 and 48 weeks as shown in Table 2.

After hospitalization, the patients were observed for 24 hr before starting the treatment. The drug was given orally in an emulsion form

TABLE 1

Diagnosis	No. of patients
Schizophrenia	18
Hypomenia	1
Degree of excitement	
++++	9
+++	7
[illegible]	3
	19

TABLE 2

Duration of excitement	No. of patients
Less than 2 weeks	7
Less than 4 weeks	2
Less than 12 weeks	7
Less than 24 weeks	2
Less than 48 weeks	1
	19

TABLE 3

Duration of treatment	No. of patients
Less than 8 days	3
Less than 15 days	14
Less than 21 days	2
	19

prepared with gum acacia, *arachis* oil and water. The drug was given initially, in dose of 12 to 24 mg. a day in equally divided doses. The dose was increased by 4–12 mg. and the maximum dose administered was between 24 and 48 mg. per day. The duration of the treatment ranged from 8 to 21 days (Table 3). Three patients had received chlorpromazine 150 mg., phenobarbitone 90 mg. and chloral hydrate 900 mg. daily for 2–3 days prior to hospitalization, without control of symptoms. Four patients received paraldehyde 8 ml. intramuscularly once daily during the first 3 days of treatment. One more patient received 7 ml. paraldehyde i.m. twice and 50 mg. of chlorpromazine parenterally twice during the treatment period. Other patients did not need any additional drugs except Harik extract.

A detailed physical and psychiatric examination of each patient was done before, during and at the termination of the drug trial. Laboratory

investigations, like complete haemogram, urine examination, liver function test, SGOT and SGPT tests were done at the beginning of the trial and repeated after one week and also at the end of the trial. Electrocardiograph studies were done at the beginning and at the end of the trial in some patients.

The changes in the patients' condition were observed by the resident physicians, the two consulting psychiatrists (L. P. Shah and V. N. Bagadia), the nursing staff, the patient's relatives, and the occupational therapist. A final assessment as to the beneficial effects of the drug was made by pooling all the information thus collected. The undesirable reactions noted were also recorded. Follow-up studies for 1–3 weeks after stopping the drug were carried out wherever possible.

RESULTS

The criteria of improvement was 50 per cent or more reduction in the excitement of these patients. An attempt was also made to observe changes in the psychotic symptomatology. The results are shown in Table 4. Majority of the patients quietened down in 2–4 days. The maximum improvement was observed in about 10 days. In 11 patients, excitement was completely controlled, 3 showed marked improvement; one showed moderate improvement, and 2 patients showed only mild improvement. Amongst the 17 patients who showed remission in excitement to a significant degree, 6 patients showed total remission in their psychotic features and one patient showed moderate remission.

Side Effects

Side effects are shown in Table 5. The main side effects observed were fine tremors, nausea, vomiting and loss of appetite. The tremors could be controlled by pacitane [2 mg. t.d.s.]. None of the side effects was severe enough to discontinue the drug treatment or necessitate reduction in dosage.

TABLE 4

No. of patients		19
Improvement		
Excitement	17	Psychotic feature 7
++++	11	6
+++	3	—
++	1	1
+	2	—
Discontinued	2	

TABLE 5

Side effects	
Tremors	13
Anorexia	13
Nausea and vomiting	12
Bradycardia	5
Tachycardia	1

Follow-up

Majority of patients relapsed within 1 to 3 weeks after discontinuing the drug. One of the patients who had relapsed after two weeks was given a second course of the treatment with Harik and he responded satisfactorily. He received the drug for a total period of 30 days.

The laboratory investigations and E.C.G. studies during and at the end of the trial did not reveal any abnormal findings attributable to the drug.

DISCUSSION

The results obtained with the present fraction of the plant extract compare identically, both in effectiveness and side effects to those observed by us in our previous study of 25 excited psychotic patients treated with dried ethanol extract obtained from Bhide. The fraction used in the present study was prepared by Deliwala, as mentioned earlier. Fraction reported by Bhide and used by Dev and also by us in previous clinical trials was an ethanol extract. The present extract was prepared by processing further the ethanol extract, as indicated above. This fraction was a buff-coloured powder stable at room temperature for a long period. The extract varied in potency from batch to batch and had to be bioassayed in cats. Degree of sedation is so variable in animals making it difficult to assess in a quantitative way the activity of a particular batch of extract. Hence another method with more definite end-point has to be selected as a parameter of activity. The criterion of activity was manifestation of mild tremors on intraperitoneal administration of the test fraction in an emulsion form. The potent fraction produced this effect at about 2 mg./kg. Subacute toxicity studies in rats, mice and dogs in a dose of 5, 10 and 20 mg./kg. given daily intraperitoneally for 6 weeks did not produce any adverse effects.

Out of the 19 patients treated, 17 improved with decrease in excitement and the patients could be easily managed within 2 to 4 days of treatment. The improvement was progressive and the maximum improvement occurred around the tenth day of the treatment and continued as long as the drug was administered. As chronic toxicity studies have not been done,

the drug could not be given for more than 2 weeks. Relapse in excitement occurred in majority of patients within 1 to 3 weeks of withdrawal of the drug. In one case, the drug was re-administered with control of excitement similar to that seen during the first course. The changes in psychotic process are noted in seven patients. It is possible that the control of excitement could be maintained if the drug is continued for a long time. A change in psychotic process could have been possible in a large number of patients, if the drug had been given over a longer period, as is commonly the practise with other major tranquillizers. The effects of this fraction resemble those obtained with major tranquillizers or what one would call antipsychotic like phenothiazines. The observed side effects like tremors and rigidity, also resemble those seen with large doses of phenothiazines and could also be controlled with antiparkinsonian drugs like pacitane. Nausea, vomiting and anorexia were troublesome side effects observed in most of the cases. However, they were never severe enough to necessitate stoppage of the treatment or reduction of the dose, or use of any antiemetic agents.

Till now we have satisfactorily treated 49 excited patients by the crude fractions obtained from this plant. Dev and Bhide and Dev reported a total of 75 patients treated with this plant extract with identical results. This extract from *Paspalum scrobiculatum* has been found to be definitely useful for controlling excitement in psychotic patients. Further work on the chemistry of this plant and chronic toxicity studies are necessary before further clinical trials over a long period could be undertaken to determine its efficacy, both in keeping under control the excitement of patients and to observe the changes in their psychotic processes.

REFERENCES

1. Bhide, N. K. & Aimen, R. A., *Nature* (Lond.), **183** (1959), 1735.
2. Bhide, N. K., *Br. J. Pharm.*, **18** (1962), 7.
3. Chanakya's *Arthashastra* (321 B.C.), English translation by Shama Sastry, 5th ed., 1956, p. 236 and 442, Raghuveer Printing Press, Mysore.
4. *Charak Samhita* (about 1000 B.C.), English translation by S. G. Ayurvedic Society, Vol. II, (1949), p. 949, Jamnagar.
5. Chevers Norman, Medical Jurisprudence in India, 3rd ed. (1870), p. 302, Thacker Spink & Co., Calcutta.
6. Dev & Bhide, *Psychopharmacologia*, **2** (1961), 295.
7. Dev, *Psychopharmacologia*, **5** (1964), 228.
8. Narhar's *Rasaghanta* (about A.D. 1400) Anandashram Sanskrit Publication No. 34 (1925), p. 225, Anandashram, Poona.
9. Shah, L. P., Doongaji, D. R., Bagadia, V. N., Vaidya, N. S. O. & Shetty, U. K., *J. Psychiatry*, **6** (1964), 131.
10. Sundaram Ayyar, K. V. & Narayanswami, K., *Nature* (Lond.), **163** (1949), 91.
11. Swarup, A., *Indian med. Gazette*, **57** (1922), 257.

Chemistry and Pharmacology of 6,7-Benzomorphans

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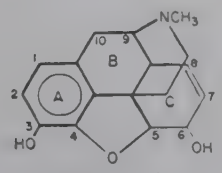
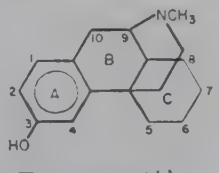
The oldest and most popular centrally acting analgesic used in the relief of moderate to severe pain is the opium alkaloid morphine (I). Ever since the chemical architecture of this drug was established with reasonable certainty, it has been the goal of many scientists, not only to totally synthesize morphine but also to fashion chemically various fragments of the molecule in the hope of separating the desirable and undesirable (particularly dependence liability) properties of this valuable drug. One of the earliest attempts at total synthesis resulted in the development of a compound, 3-hydroxy-*N*-methylmorphinan (II) containing the complete carbon-nitrogen skeleton of morphine but lacking such peripheral groups as the oxygen bridge, the allylic hydroxyl and the alicyclic double bond. Despite this simplification, the *levo* isomer of this substance (trade-named *levo*-Dromoran and with the international nonproprietary name levorphanol) is some four times as potent on a dosage basis as morphine with no greater side effects, including dependence liability, than morphine. The 6, 7-benzomorphans (III), the subject of this presentation, represent a still further abbreviation of the molecule with, in many instances, enhancement of activity and with elimination or minimization of some of the more harmful effects of morphine and levorphanol as will be pointed out later.

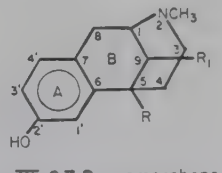
In essence, the 6, 7-benzomorphans (III, R=alkyl, R_1=H or alkyl) are derived simply by deleting 2 or 3 carbons of ring C of levorphanol. In other words, morphine and levorphanol are asymmetric octahydrophenanthrenes while the benzomorphans are tetrahydronaphthalenes. All three



I Morphine (-)



II Levorphanol (-)

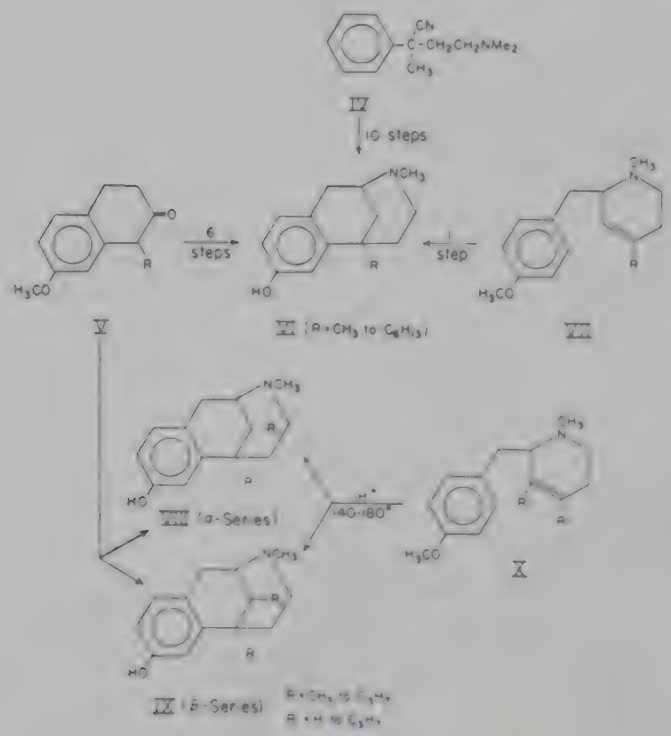


III 6,7-Benzomorphans

have an N-methyliminoethano system (or furyl in the case relative position and other features in common. The portions of morphine and levorphanol duplicated by the 6, 7-benzomorphans are indicated by heavy lines.

Initially, simple model benzomorphans were synthesized and evaluated in animals. These showed enough CNS activity (analgesic and conditioned-response-blocking) to warrant further research. Consequently, from about 1955–63 some 50 or 60 new benzomorphans were synthesized by our group at the National Institute of Health in a variety of ways. Their stereochemistry was determined relative to morphine and levorphanol at the three common centres of asymmetry. Only the highlights of these syntheses and structure elucidations can be presented within a reasonable compass.

In the earliest stages of the research, either a phenyl-acetonitrile derivative (IV) or 1-methyl-3-tetralone (V) were the starting materials. With (IV) as the base point a 6-membered carbocyclic ring evolving from the nitrile portion of the molecule and a heterocyclic ring (also 6-membered) arising from the dimethylaminoethyl side-chain had to be fused to the benzene ring. Construction of the nitrogenous ring only was necessary when (V) was used. The integrity of quaternary carbon is assured by the C-methyl substituent in each instance. Later, as the work progressed, a more convenient and versatile method was sought and in a modification of the Grewe morphinan synthesis. A 1-benzyloctahydroisoquinoline was the immediate precursor of the morphinans, while for the 6,7-benzomorphans, appropriately 3, 4-dialkyl substituted 2-benzyl-1-methyl-1, 2, 5, 6-tetrahydropyridines were cyclized in acid medium to the desired products. In this way several



homologous 6, 7-benzomorphans have been prepared and evaluated for analgesic, physical dependence and conditioned-response-blocking properties.

Because there are three asymmetric carbons (at positions 1, 5, and 9) in the final products, 4-racemates or 8 optically active isomers are theoretically possible. However, the iminoethano system is geometrically constrained to a *cis*-diaxial fusion at positions 1 and 5. This restriction eliminates 2 racemates. Invariably both possible racemates were obtained in a ratio as high as 10 : 1 or as low as 2 : 1, depending upon the cyclization conditions and the bulk of R and R_1. That the two products (VIII and IX) are isomeric at position 9 was shown by their degradation to the same 7-methoxy-1, 2-dialkylnaphthalene in 3 series. The stereochemistry of the predominant α-isomers (VIII) was proved to be the same as that of morphine and the morphinans (levorphanol) at the three common centres of asymmetry while the β-isomers (*trans*-juxtaposition of R and R_1) was linked with the isomorphinans, first obtained as an intermediate in one of the several total syntheses of morphine.

The α-isomers (by subcutaneous administration) are also comparable to morphine and levorphanol (II, a morphinan) in analgesic activity and acute toxicity but proved to be unique in having greatly reduced or no physical dependence capacity (PDC) (i.e. the capacity to support a morphine addiction) in monkeys in contrast to morphine, levorphanol, pethidine and other strong analgesics. The β-isomers were 7–70 times more potent as analgesics than the α-counterparts but were also somewhat more toxic and addicting. In Table 1 are shown analgesic activity, acute toxicity and PDC of several- 5-alkyl and α- and β-5, 9-dialkyl-2′-hydroxy-2-methyl-6, 7-benzomorphans. As can be observed, peak analgesic activity is displayed in all three series when the carbon total at positions 5 and 9 is 2-4.

Because of their lower toxicity and little PDC, compounds of the α-series were chosen for further study. As is well known, the compounds

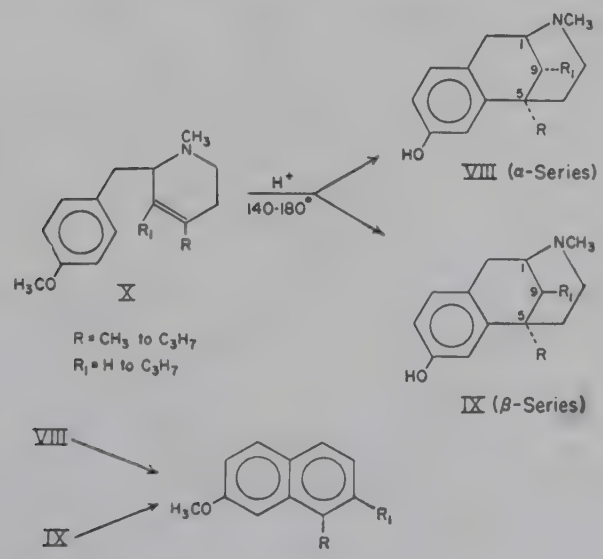
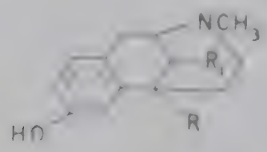
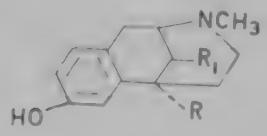


TABLE I—PHARMACOLOGY OF (+)-5-ALKYL AND (+)-5, 9-DIALKYL-2-METHYL-6, 7-BENZOMORPHANS



α-5-Monoalkyl and α-5, 9-dialkyl compounds

No.	R	R_1	ED_{50}	LD_{50}	Abstinence suppressant dose mg./kg.	Physical dependence capacity
1.	Me	H	10·4	175	2-60, No suppression	None
2.	Et	H	2·3	170	1-16, No suppression	Low
3.	Pr	H	2·1	130	3-30, No suppression	None
4.	Bu	H	2·0	130		
5.	Am	H	3·4	93		
6.	Hex	H	10·8			
7.	Me	Me	3·0	175	24	Low
8.	Me	Et	1·5	134	2-12, No suppression	None
9.	Et	Me	4·9	309	>40	Low
10.	Et	Et	4·2	425	2-60, No suppression	None
11.	Pr	Me	2·9	>300		
12.	Pr	Pr	71·2	>400	3-48, No suppression	None



β-5, 9-Dialkyl compounds

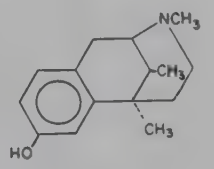
13.	Me	Me	0·44	67	>18	Low
14.	Me	Et	0·47	100		
15.	Et	Me	0·07	75	1·0	Intermediate
16.	Et	Et	0·28	120	0·5-12, No suppression	None
17.	Pr	Pr	0·87	55		
	Morphine		2·1	550	3	High

obtained by chemical synthesis are racemates. It is also well known that all the analgesic activity of (+)-3-hydroxy-*N*-methylmorphinan is due to the levorotatory antipode and that morphine is levorotatory. Consequently, in an attempt to improve efficacy one of the most interesting racemates (compound 7 of Table I) was resolved into its enantiomers. Like 3-hydroxy-*N*-methylmorphan, all of the analgesic activity and little of the acute toxicity

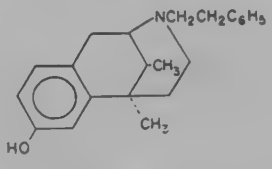
of this racemate was elicited by the *levo*-isomer which was found to be as effective as morphine for pain relief in animals and in men. This *levo* compound had no PDC in monkeys in single-dose suppression experiments and when tested in human post-addicts, a unique and heretofore unnoticed separation of morphine-like effects was observed. Thus, it was equivalent to morphine in producing the usual, morphine-like subjective effects but was little better than a placebo when substituted for morphine in an established addiction. In other words, it was little effective in controlling withdrawal symptoms. Furthermore, the development of tolerance was slower and abstinence symptoms less than half as severe with this drug than with morphine upon 30-day chronic administration.

An even better separation (at least in animal species) of favourable and adverse effects has been observed on resolution in the 5, 9-diethyl series (compound 9 of Table 1). In this instance the *levo*-isomer is less toxic, more than twice as potent as the racemate (in this respect morphine-like) and will not support morphine physical dependence in monkeys. In contrast, the *dextro*-isomer, 1/8 as potent as the *levo*-isomer (codeine-like), was reasonably effective in substituting for morphine in dependent monkeys. There are other indications of antagonism between these optical opposites, but these must await confirmation.

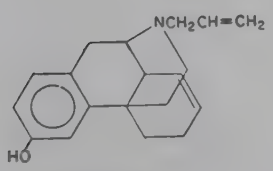
So far, we have dealt with compounds having a methyl substituent on the heterocyclic nitrogen. In fact, until 1955, it had been the consensus that this group has an optimal structural feature for strong analgesia. About this time a research team in the United States (Merck & Company), while examining a large group of *N*-substituted morphine congeners for nalorphine-like antagonistic activity demonstrated that *N*-phenethylnormorphine was six times more potent analgesic than the parent morphine. Naturally, this exciting result stimulated research in other series regarding changes at the nitrogen function. We again decided to operate on the racemate described before α-(±)-5, 9-dimethyl-2′-hydroxy-2-methyl-6, 7-benzomorphan (XI). Substituting phenethyl for methyl caused a 3 to 8-fold increase in potency. The



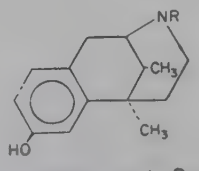
XI



Phenazocine
(Prinadol, Narphen)



Nalorphine
(Nalline)



R = $CH_2CH=C(CH_3)_2$-Pentazocine

R = $CH_2{}^{\Delta}$-Cyclazocine

resulting compound with the international nonproprietary name pheniazocine is now sold under such trade names as Narphen and Prinadol. It is a very effective analgesic in man with fewer and less harmful side effects, including lowered abuse liability than morphine, at optimal doses. It is also much more effective for oral use than morphine; in fact, it has proved to be very advantageous for chronic pain by this route up to two-year periods without apparent development of tolerance and physical dependence.

It was also about 1955 that the first report appeared of the analgesic effectiveness of the antagonist nalorphine (N-allylnormorphine). Despite its inadequacy in animal pain (and lack of abuse liability in man) it was shown to be as effective as morphine mg. for mg. in relieving human suffering. However, nalorphine's bizarre side effects, mostly of a psychotomimetic nature, at the analgesic dose, forbade its use as a practical analgesic.

Based on these findings and those just described for the benzomorphans, scientists of the Sterling-Winthrop Laboratories, US, have varied the group on the nitrogen of the basic 5, 9-dimethyl-2′-hydroxy-6, 7-benzomorphan in such a way as to give mild to very potent antagonists. While most of these compounds were inert or of a low order of activity in animal species two were potent analgesics in man. One of these, pentazocine, the N, N-dimethylallyl analog, is a weak antagonist of morphine-like actions, and a dose of 20–40 mg. appears to be nearly as effective as 10 mg. of morphine for pain relief in humans. Side-actions, including respiratory depression, are similar to those of morphine, but pentazocine's abuse liability is nil. It produces mild psychotomimetic effects at higher doses similar to, though milder than, those of nalorphine. It appears to be the first potent analgesic, without dependence liability to be suitable for clinical use. The other, cyclazocine, the cyclopropylmethyl derivative, is a very powerful antagonist to morphine and similar narcotics. Either orally or parenterally, 0·25 mg. of this compound is equivalent to 10 mg. of morphine in post-operative pain. This dose has produced no nalorphine-like subjective effects. It does elicit a mild, but a typical abstinence syndrome after prolonged chronic administration, more nalorphine-like than morphine-like. It has a long duration of action. Tolerance develops to the subjective effects of cyclazocine but not to its ability to antagonize almost all the pharmacologic actions of morphine, including its euphoria-producing property. When administered twice daily, orally, in doses of 2 mg. 70 kg., cyclazocine will antagonize the euphoria-producing effects of very large doses of morphine as well as the development of physical dependence to morphine. Its use for the ambulatory treatment-management of addicts who are highly motivated to remain off narcotics has been recommended.

Most of the 6, 7-benzomorphans described, elicited conditioned-response-blocking activity in mice and rats. However, this activity is somewhat erratic and it would be difficult to correlate this property with analgesic activity or physical dependence property.

In summary, the 6, 7-benzomorphans represent a new class of CNS agents simpler in structure than the prototypes morphine and the morphinans but equally effective or more so, in the relief of moderate to severe pain. There is little doubt that, in this class, appreciable separation of desirable and undesirable medical properties has been achieved. In a few instances a virtually complete divorcement of strong analgesic activity and dependence liability (particularly in animal species) is seen.

Action of Some Microsomal Inhibitors on the Effects of Morphine and Related Analgesics

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Drugs which inhibit the metabolic inactivation of analgesics potentiate their effects. Carbon tetrachloride (CCl_4) was found to potentiate the analgesic effect of morphine and of some related drugs (Komlos and Komlos-Szasz, 1954; Herken *et al.*, 1959) and to abolish tolerance to the analgesic effect of morphine in rats (Komlos, 1956). This effect of CCl_4 is thought to be elicited by an inhibitory action on the microsomal enzymes in the liver, whereby the rate of the metabolic inactivation of analgesics can be decreased. The enzymes which are involved in the metabolic transformation of analgesic drugs are thought to be present in the hepatic microsomes of various animal species (Bernheim and Bernheim, 1944, 1945; Adler and Shaw, 1952; Hoffmann *et al.*, 1955; Axelrod, 1956; Cochin and Axelrod, 1957, 1959; Mannering and Takemori, 1959; Herken *et al.*, 1959; Takemori, 1960; Roth and Bükowsky, 1961).

However, the mode of action of CCl_4 upon analgesic drugs is still obscure since possibility has been announced that CCl_4 might exert its effect not only by its hepatotoxic action (Herken *et al.*, 1959). The opinion has been expressed several times that the phenomenon of tolerance is connected with the changes in the metabolic transformations which ensue when analgesic drugs are repeatedly applied. Thus a better understanding of the mode of action of drugs such as microsomal inhibitors, which affect the metabolic transformation of analgesics, might contribute to an improvement of our knowledge concerning tolerance to drugs.

The experiments presented here, were undertaken to study the possibility that the extra-hepatic mechanism(s) is (are) involved in the potentiating action of the so-called microsomal inhibitors.

METHODS

The analgesic action was studied on albino rats (groups of 10 animals) of both sexes, weighing 150–200 g. Analgesia was tested according to the method of D'Amour and Smith (1941). That is, a beam from a 50 W. bulb, supplied by a 12 V. battery, was focussed on the tip of the rat's tail. Before the injection of the analgesic drug the control measurements were done at 15–20 min. intervals, during a control period of 1 to 1·5 hr. The dissipation of the control reaction times was thereby minimized. In order to avoid burning of the tails, the period of stimulation by heat was limited to approximately 14 sec. (mean control reaction time, which amounted to 4, 5·5 sec. plus twice this value, i.e. 9 sec.). The animals which did not show the characteristic movement of the tail in this period of stimulation were recorded as having 'total analgesia'. The same groups of rats were injected with CCl_4 7 to 10 days later and thereafter with the analgesic drug. Thus, each group of rats was tested twice for analgesia, the first testing representing the control experiment. The analgesic drugs were injected intraperitoneally, and CCl_4 subcutaneously (2·5 to 3 ml./kg.).

In experiments on mice, analgesia was tested according to Woolfe and Macdonald (1944). Each mouse was placed on a hot plate (53°C.) and the reaction time until the paw licking reflex was recorded. Only those mice which responded in 10 to 15 sec. were used and were divided into groups of ten animals. In order to prevent damage to the paw by heat, mice which did not respond in the period of double the mean control reaction time ('cut-off time'), were removed from the plate. Thus 50 per cent cut-off time in the graphs corresponds to the control mean reaction time.

Intracerebral injections were given according to Matthies and Schmidt (1961), into the hind brain, 2 mm. deep, with fine intradermal needle, the drugs dissolved in saline. The total volume of the solution injected intracerebrally was arranged to amount to 0·01 ml.

Cordotomy was performed on rats of both sexes under aseptic conditions and with ether anaesthesia. A part of spinal cord in the Th V region was operatively removed. Only those animals which showed positive spinal reflex after the operation, were used in the experiments. Urine had to be expressed in regular intervals in operated animals. Five to 9 days later the animals were used for testing the effects of drugs (according to D'Amour and Smith, 1941).

The *in vitro* experiments were done on isolated guinea-pig ileum, which was suspended in magnesium-free tyrode solution, kept at 36°C. and bubbled with O_2. For the electrical stimulation two platine wire electrodes were used, one of them being placed intraluminally and the other one being immersed in the bathing fluid. The rectangular pulses were obtained from a pulse generator. The series of 5 pulses per sec. (0·2 msec., 10V.) were directed to the gut during 5 sec. in 30 to 60 sec. periods. CCl_4, which is only slightly

soluble, was added in excess to the bathing fluid by means of pledgets of cotton wool.

The antidiuretic effect of analgesics was examined on rats of both sexes, weighing 150 to 200 g. The animals were divided into groups of 10, one of the groups receiving saline instead of the analgesic drug. These drugs were given subcutaneously or intraperitoneally. CCl_4 (3 ml./kg.) was injected subcutaneously 48 hr before the analgesic drug. During the pre-experimental period care was taken to allow a steady access to water. On the day of the experiment, a water load was given to each rat (6 ml./100 g. of body weight) orally, by duodenal tube. Immediately thereafter the analgesic drug was injected. Urine was collected and the volume measured at regular intervals. The results were expressed graphically and the time needed for excretion of 50 per cent of the water load was determined.

RESULTS

Action of CCl_4 on the Analgesic Effect of Morphine, Dihydromorphinone, Methadone, Codeine and Pethidine

The pretreatment of rats by CCl_4 potentiated the analgesic effect of morphine. The same dose of morphine (4 mg. kg.$^{-1}$), when given before CCl_4, elicited analgesia which culminated 35 min. after the injection and lasted for 95 min. The 'mean reaction time' of the control period was considerably increased, but all the animals showed the typical reaction to heat stimulus. Thus, none of them had 'total analgesia'. Thereafter the same group of animals received CCl_4 and, 48 hr later, morphine was injected. Other groups of rats were given morphine 3, 4 and 5 days respectively after CCl_4. In each of the CCl_4 pretreated groups the analgesic effect of morphine was potentiated and prolonged. However, the influence of CCl_4 was most obvious 48 hr after pretreatment. In this group, morphine elicited 'complete analgesia' in 6 out of 10 rats, and the mean reaction time was increased by 6·2 sec. The duration of analgesia was doubled. CCl_4 produced a similar action in experiments in which dihydromorphinone and methadone were used as analgesics. The effects of both drugs were potentiated and prolonged by CCl_4 pretreatment. In the experiment with dihydromorphinone, the number of rats showing total analgesia at the peak of the effect was increased from 3 out of 10 (in the group untreated with CCl_4) to 8 out of 10 (in the CCl_4 pretreated group). When methadone was used the number of rats showing total analgesia amounted to 1 in CCl_4 untreated group and 5 in CCl_4 treated group.

The analgesic effect of pethidine was potentiated by CCl_4 pretreatment. However, the duration of analgesia elicited by pethidine was not substantially changed by treating the rats with CCl_4. In both groups of rats the peak of the analgesic effect was already achieved 15 min. after the injection of pethidine (15 mg. kg.$^{-1}$). In the control, CCl_4 untreated group, 5 out of 10 rats showed total analgesia at the peak of the effect. The number of

animals showing total analgesia was increased to 8 by CCl_4 pretreatment. The influence of CCl_4 on the analgesic effect of codeine was studied by using three doses of the analgesic. The first, small dose of codeine was inefficient, while the remaining two doses elicited a clear-cut effect. The pretreatment of rats by CCl_4 48 hr before the analgesic considerably potentiated its analgesic effect. Furthermore, the small previously inefficient dose of 4 mg. kg. of codeine elicited analgesia when used 48 hr after CCl_4.

The influence of CCl_4 on the analgesic effect of the two higher doses of codeine 8 mg./kg. and 20 mg./kg. was thereafter compared with the potentiation which CCl_4 pretreatment caused in the effect of two doses of morphine, which were found to be equipotent with the two doses of codeine. That is, the effect of 8 mg./kg. of codeine corresponded well with the effect of 4 mg./kg. of morphine, and a comparable degree of analgesia was produced by the dose of 10 mg./kg. of morphine and 20 mg./kg. of codeine. A high proportion of animals showing total analgesia was found especially in CCl_4 pretreated groups and with higher doses of analgesics. Since this enabled a precise comparison of the effects, the duration of the analgesia was used as the criterion. Therefore, the time during which 50 per cent analgesia was lost was determined for both CCl_4 untreated as well as CCl_4 treated groups. The results have shown that the effects of smaller equiactive doses of morphine and codeine were equally potentiated by CCl_4. However, the effect of the high dose of codeine was potentiated by CCl_4 pretreatment more than the effect of the previously equiactive dose of morphine. Thus the effect of 20 mg./kg. of codeine was potentiated 1·9 times more than the effect of 10 mg./kg. morphine.

Influence of Cordotomy on the Potentiating Action of CCl_4 on the Effect of Morphine

The reactivity to heat stimulus of cordotomized rats was first examined. The movement of the tail in these animals was slow, but the reaction times were shortened as compared with those in normal rats. Morphine elicited analgesia, but the effect was less marked than in normal animals. In cordotomized rats pretreatment with CCl_4 did not change the reaction times. However, the analgesic effect of morphine (10 mg./kg.) in such animals was potentiated by CCl_4 pretreatment given 48 hr before morphine. A comparison of the potentiation by CCl_4 obtained in cordotomized animals with that obtained in normal rats is difficult, owing to the difficulties already described which result from the actual method used. However, the impression was gained that less potentiation by CCl_4 was obtained in cordotomized than in normal rats.

Action of CCl_4 on the Antidiuretic Effect of Morphine and Codeine

In these experiments CCl_4 was found to be without effect on the excretion of the waterload in rats. Morphine (10 mg./kg.) and codeine

(20 mg./kg.) both elicited an antidiuretic effect with both intraperitoneal and subcutaneous injections. The doses of analgesic drugs were chosen so as to be equipotent. The dissipation of the individual records around the mean was less pronounced after intraperitoneal injection than after the subcutaneous application of the drugs. In the experimental groups which were treated with CCl_4 48 hr before the analgesic drug, the antidiuretic effects were not potentiated. These effects were antagonized by CCl_4 pretreatment, the influence of CCl_4 being more pronounced in the groups which received the analgesics by the intraperitoneal route.

Action of CCl_4 on the Effect of Morphine on the Isolated Guinea-pig Ileum

Isolated guinea-pig ileum is thought to be a paradigm suitable for the analysis of drugs which act on the CNS (Schaumann *et al.*, 1952, 1953a, 1953b; Schaumann, W., 1955; Paton, 1957; Medakovic, 1958). Thus, experiments on such an isolated organ offer an analysis of the influence of CCl_4 on the effect of morphine, without the presence of the hepatic mechanisms which are responsible for the metabolism of analgesics.

In these experiments, the findings of Paton (1957) were confirmed, since morphine inhibited the twitch of the isolated gut, stimulated electrically. The efficient concentrations of morphine were as low as 10^{-9} g./ml. CCl_4 alone was without effect, but when morphine was added in the presence of CCl_4, or *vice versa*, a more pronounced depression of the twitch was obtained than by morphine alone.

With repeated additions of morphine into the bath, the effect of this drug on the twitch tended to decrease. The same thing was observed after a higher dose of morphine. In both instances a state was produced wherein the previously efficient concentrations of morphine were without effect on the twitch. However, the efficiency of the same small concentration of morphine was re-established in the presence of CCl_4. Thus CCl_4 was able to abolish the phenomenon which is called 'acute tolerance' (Paton, 1957).

Action of CFT Compounds on the Effect of Morphine in Mice

First, the effect of morphine injected intracerebrally was studied. The reaction times of mice to heat stimulus were found to be prolonged after the intracerebral injection of morphine. This effect of morphine was dose-dependent. The dose of 0·5 mcg. of morphine was chosen for further study.

In the second experiment, two groups of mice received CFT compounds intraperitoneally. Compound CFT 1201 was injected into one group and the compound CFT 1208 into the other one. The third, the control group, received saline intraperitoneally. Morphine was injected intracerebrally 90 min. after the CFT compound (or saline). Both CFT compounds were found to potentiate the analgesic effect of morphine as compared with the

effect of this analgesic in the control group. The potentiation was higher with CFT 1201 than with 1208.

It should be mentioned that in the control period of each experiment, before the injection of morphine, the reaction time to the heat was moderately and briefly prolonged in the group of mice receiving CFT 1201. The maximum of this effect was recorded 15 min. after the intraperitoneal injection of the compound, but it was insignificant at the time of morphine injection.

It should also be pointed out that the real potentiation of the morphine effect by the CFT compounds could not be adequately recorded. That is, in the group of mice pretreated with CFT 1201, cut-off time was recorded in 8 out of 10 mice. This means that the real prolongation of the reaction time in this group was much higher than that taken for the comparison with the effect of morphine in the control group. Also, the true potentiation of the morphine analgesia with the compound CFT 1208 must have been higher than could be recorded, since in this very group, in 5 out of ten mice total analgesia was found. On the other hand, none of the mice in the control group receiving morphine without CFT compound had total analgesia.

The same drugs were used with reversed routes of injection in the subsequent experiment. Thus, the CFT compound (CFT 1201 or CFT 1208) was injected intracerebrally (50 mcg.) into two respective groups of mice. The third group received saline. Morphine was injected subcutaneously 80 min. later. Both CFT compounds injected intracerebrally were found to potentiate the effect of subcutaneously injected morphine. Owing to the high rate of mice showing total analgesia in groups pretreated with CFT compounds, the real potentiation of the morphine action was not recorded in this experiment either. This must have been much higher than could be recorded.

It should be mentioned that immediately after intracerebral injection, signs of central stimulation were observed. The animals appeared quiet but, on being touched, started running and some had convulsions. None of them died, but those animals which had convulsions were rejected.

DISCUSSION

The main goal of the experiments presented here was to explore the mode of analgesia potentiating action of the so-called microsomal inhibitors.

One of these microsomal inhibitors, carbon tetrachloride, was found to potentiate the analgesic effect of all the analgesics tested in the present experiments on rat. The effects of morphine, methadone, dihydromorphinone and codeine were both potentiated and prolonged, while the effect of pethidine was only potentiated. These findings indicate the mode of the analgesia potentiating action of CCl_4.

Two processes were found to be essential for the fate of analgesic drugs: dealkylation and conjugation. The latter process is thought to be more important than the former for the metabolic inactivation of morphine (Richter,

1951; Zauder, 1952; Adler and Shaw, 1952; Adler *et al.*, 1955; Herken *et al.*, 1955). On the other hand, pethidine was found to be demethylated more than the other analgesic drugs (Axelrod, 1956) and conjugation is not important for the metabolic inactivation of this analgesic (Pienaar *et al.*, 1956).

It is reasonable to expect that by inhibiting the main process of the metabolic inhibition of a drug, its effect should be both potentiated and prolonged. This was found to be valid in the present experiments for morphine, methadone, dihydromorphinone and codeine, which is in accordance with our expectations. Also the potentiation of the effect of pethidine without prolongation would agree with the views that pethidine is mostly demethylated. This suggests that CCl_4 does not considerably inhibit demethylation.

Thus, the mode of potentiation of the effect of pethidine by CCl_4 remained without a feasible explanation. It seemed that in this potentiation some rather different mechanism than the inhibition of the hepatic microsomal enzymes was implied. The possibility that, besides the inhibition of the metabolic degradation in the liver, some other mode of action of CCl_4 might be involved in its influence on the analgesic effect of morphine and the related drugs, was tested in the subsequent experiments.

First, the influence of CCl_4 on another, nonanalgesic, action of analgesics was studied. The antidiuretic action of morphine and codeine was chosen for this purpose. It was found that this action of morphine and of codeine was not potentiated by CCl_4, irrespective of the route of the injection of the analgesic drugs (intraperitoneal or subcutaneous). Furthermore, the antidiuretic effect of both analgesics was somewhat inhibited by CCl_4 pretreatment. This finding agrees well with the fact that SKF 525A, which is another inhibitor of the hepatic microsomal enzymes, potentiated the analgesic effect of morphine, but failed to affect the toxicity (Cook *et al.*, 1953; Swinyard *et al.*, 1954) and the sedative stupor-inducing action of morphine (Herz, 1961). Thus a working hypothesis was formulated that there are probably some other more specific mechanisms by which the analgesic effect of morphine and related drugs might be potentiated by CCl_4. The possible mechanisms were studied in further experiments, which were performed on cordotomized rats, on isolated guinea-pig ileum and by means of a combination of routes of injection of both the analgesic drug and the potentiating agent. It has been previously shown (Brody, 1959, 1961) that cordotomy can abolish the action of CCl_4 on rat's liver. Although the mode of this action of CCl_4 was much discussed (Judah and Rees, 1959; Larson and Plaa, 1965) the original findings of Brody were generally accepted. In the present experiments cordotomy did not abolish the tail twitch to stimulation by heat, but the reaction was changed in some respects: the reaction times were shortened and the movement of the tail was slow. In view of the fact that in these animals the spinal cord was transected, the actual movement of the tail in the presence of heat can be only a spinal

reflex. This was found to be inhibited by morphine and the effect of morphine was potentiated by CCl_4. The control experiments, concerning the activity of the liver microsomal enzymes of CCl_4 treated cordotomized rats, were not performed in the present experiments. Therefore, the results of the experiments performed on cordotomized rats had to be evaluated in some other way. At that very moment, the introduction of a suitable model in the further analysis of the mode of the potentiating action of CCl_4 seemed promising. Isolated guinea-pig ileum was chosen for this purpose because, this isolated organ has been already found to represent a paradigm for the analysis of the action of drugs which act upon the CNS. In the present experiments, CCl_4 was found to potentiate the inhibitory action of morphine on the twitch of the electrically stimulated gut. Furthermore, the 'acute tolerance' to morphine, which was caused by the repeated addition of morphine into the bathing fluid, was also abolished by CCl_4. This latter finding can be connected with the results of Komlos (1956), who has shown that CCl_4 can abolish tolerance to morphine in rats, *in vivo*.

Nevertheless, the findings of the present *in vitro* experiments argue in favour of an extra-hepatic mode of the potentiating action of CCl_4 on the effect of analgesics. Since these results were obtained with a preparation which is thought to be a paradigm of the more complicated central nervous network, the possibility had to be taken into account that the very extra-hepatic action of CCl_4 is exerted on the central nervous pathway.

Therefore, experiments were undertaken in which drugs, analgesics as well as the 'hepatic microsomal inhibitors' were injected intracerebrally. In the first experiment on mice, CFT compounds, injected intraperitoneally, potentiated the analgesic effect of morphine, injected intracerebrally. The dose of intracerebrally injected morphine amounted to only 0·5 mcg., and it can be hardly expected that the fate of the part of the injected dose which could escape into the blood stream and reach the liver, could be of any significance for the potentiation of the analgesic effect of morphine by CFT compounds. In the subsequent experiment the reverse route of the injection of the drugs was followed: CFT compounds were injected intracerebrally, and morphine subcutaneously. The effect of morphine was potentiated by the pretreatment with CFT compounds in this experiment also. Here too, it can hardly be expected that any considerable amount of CFT compound could escape into the blood stream so as to inhibit the hepatic enzymes. To achieve the inhibition of hepatic enzymes a very high dosage is needed, amounting to approximately 2000 mcg. per mouse (Maibauer *et al.*, 1958), and in the present experiment only 50 mcg. of CFT compounds, injected intracerebrally was sufficient to potentiate the effect of morphine.

According to the data presented here, the conclusion seems justified that the so-called hepatic microsomal enzyme inhibitors affect the action of morphine-like analgesics not only by the action on the liver. Another mode of the action of these enzymic inhibitors is probably involved in their

potentiation of the effect of analgesic drugs. The central nervous system seems to be the site of this extra-hepatic component.

The compound CFT 1201 has a higher activity on the microsomal enzymes than the compound CFT 1200 (Maibauer *et al.*, 1958). Therefore the fact that the same compound CFT 1201 was also more active in potentiating the effect of morphine, by a mechanism which probably involved the brain, deserves attention. The view has already been expressed that the metabolic changes which a molecule of morphine undergoes in the liver may be a model for the processes which occur in the receptors of morphine action in the brain (Axelrod, 1956). The *in vivo* transformation of morphine in the brain of rats (Milters, 1962) as well as that of pethidine by rat brain tissue *in vitro* (Herken *et al.*, 1959) was also demonstrated. This is well in accordance with the hypothesis that the metabolic transformation of analgesic drugs in the brain is involved in the analgesic effect (Beckett *et al.*, 1956). In connection with these facts, the present results suggest that the use of inhibitors of the metabolic transformation of analgesic drugs might represent an attractive approach to the study of the sequence of chemical reactions leading to analgesia, as well as to the phenomenon of tolerance.

REFERENCES

1. Adler, T. & Shaw, F. H., *J. Pharmac.*, **104** (1952), 1.
2. Adler, T., Fujimoto, I. M., Way, L. E. & Baker, E. M., *ibid.*, **114** (1955), 251.
3. Axelrod, J., *ibid.*, **115** (1956), 259.
4. Beckett, A. H., Casy, A. F. & Harper, N. J., *J. Pharm. Pharmac.*, **8** (1956), 874.
5. Bernheim, F. & Bernheim, M. L. C., *J. Pharmac.*, **81** (1944), 374.
6. Bernheim, F. & Bernheim, M. L. C., *ibid.*, **83** (1945), 85.
7. Brody, T. M., *Fed. Proc.*, **18** (1959), 1017.
8. Brody, T. M., Calvert, D. N. & Schneider, A. F., *J. Pharmac.*, **131** (1961), 341.
9. Cochin, J. & Axelrod, J., *ibid.*, **119** (1957), 139.
10. Cochin, J. & Axelrod, J., *ibid.*, **125** (1959), 105.
11. Cook, L., Navis, G. G., Tonner, L. & Fellows, E. J., *Fed. Proc.*, **12** (1953), 299.
12. D'Amour, F. F. & Smith, D. L., *J. Pharmac.*, **72** (1941), 74.
13. Herken, H., Neubert, D. & Timmler, R., *Arch. exp. Path. Pharmak.*, **237** (1959), 379.
14. Herz, A., *ibid.*, **241** (1961), 236.
15. Hoffmann, J., Himes, M. B., Lapan, S., Riszki, R. & Post, J., *Arch. Pap.*, **59** (1956), 429.
16. Judah, J. D. & Rees, K. R., *Fed. Proc.*, **18** (1959), 1013.
17. Komlos, E., *Acta physiol. hung.*, **9** (1956), 261.
18. Komlos, E. & Komlos-Szasz, V., *ibid.*, **6** (1954), 451.
19. Larson, E. R. & Plaa, G. L., *J. Pharmac.*, **147** (1965), 103.
20. Maibauer, D., Neubert, D. & Rothka, H., *Arch. exp. Path. Pharmak.*, **234** (1958), 474.
21. Mannering, G. J. & Takemori, A. E., *J. Pharmac.*, **127** (1959), 187.

Compound CFT 1201 (Chemische Fabrik Tengelhof) is diethylaminoethylphenyldiallyl acetate hydrochloride and CFT 1200 is diethylaminoethylphenylallyl acetate hydrochloride.

22. Matthies, H. & Schmidt, J., *Arch. exp. Path. Pharmak.*, **241** (1961), 508.
23. Medakovic, M., *Arch. int. Pharmacodyn.*, **114** (1958), 201.
24. Milters, K., *Acta pharmac. tox. Kobenhavn*, **19** (1962), 235.
25. Paton, W. D. M., *Br. J. Pharmac.*, **12** (1957), 119.
26. Plotnikoff, N. P., Way, L. E. & Elliot, H. W., *J. Pharmac.*, **117** (1956), 414.
27. Richter, M. L., *J. Pharmac.*, **102** (1951), 94.
28. Roth, J. S. & Bukowsky, J., *ibid.*, **131** (1961), 275.
29. Schaumann, O., Giovannini, M. & Jochum, K., *Arch. exp. Path. Pharmac.*, **215** (1952), 460.
30. Schaumann, O., Jochum, K. & Schaumann, W., *ibid.*, **217** (1953a) 360.
31. Schaumann, O., Jochum, K. & Schmidt, N., *ibid.*, **219** (1953b), 302.
32. Schaumann, W., *Br. J. Pharmac.*, **10** (1955), 456.
33. Swinyard, E. A., Madsen, J. A. & Goodman, L. S., *J. Pharmac.*, **111** (1954), 54.
34. Takemori, A. E., *ibid.*, **130** (1960), 370.
35. Woolfe, G. & Macdonald, A. D., *ibid.*, **80** (1944), 300.
36. Zauder, H. L., *ibid.*, **104** (1952), 11.

6-Aminoquinazolinones as Potent Analgetic Agents

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Our continuing interest in the analgetic field and the report of Kacker and Zaheer[1], prompted us to prepare a series of amino-substituted 4-quinazolinones. The compounds of these workers proved to have hypnotic activity[2] and possibly acted as a disincentive to further study as analgetic agents. We have found in our laboratories that the introduction of an amino group in the 6-position of the 4-quinazolinone structure enhances the pharmacodynamic activity in general and analgetic activity in particular. The results of the pharmacological evaluation of these compounds will be discussed.

Most of these compounds were prepared by the general method (A)[3] in which a substituted *o*-acylamido benzoic acid is condensed with an aromatic amine in the presence of phosphorus trichloride. Some of the compounds were prepared by method (B)[4] where a substituted isotoic anhydride was treated with an excess of aromatic amine to give the corresponding anthranilamide. The amide was treated with an appropriate organic monocarboxylic acid preferably with heating to produce the cyclized nitro intermediate which was catalytically reduced to give the desired product.

A general scheme and example for methods A and B are given in Fig. 1.

EXPERIMENTAL

All melting points were recorded on a Thomas-Hoover Unimelt Capillary melting point apparatus and are uncorrected. The reported overall yields were based frequently on one run and do not necessarily reflect the optimum yields obtainable. Microanalyses were performed by Schwarzkopf Microanalytical Laboratories, Woodside, New York, USA and or Galbraith Microanalytical Laboratories, Knoxville, Tennessee,

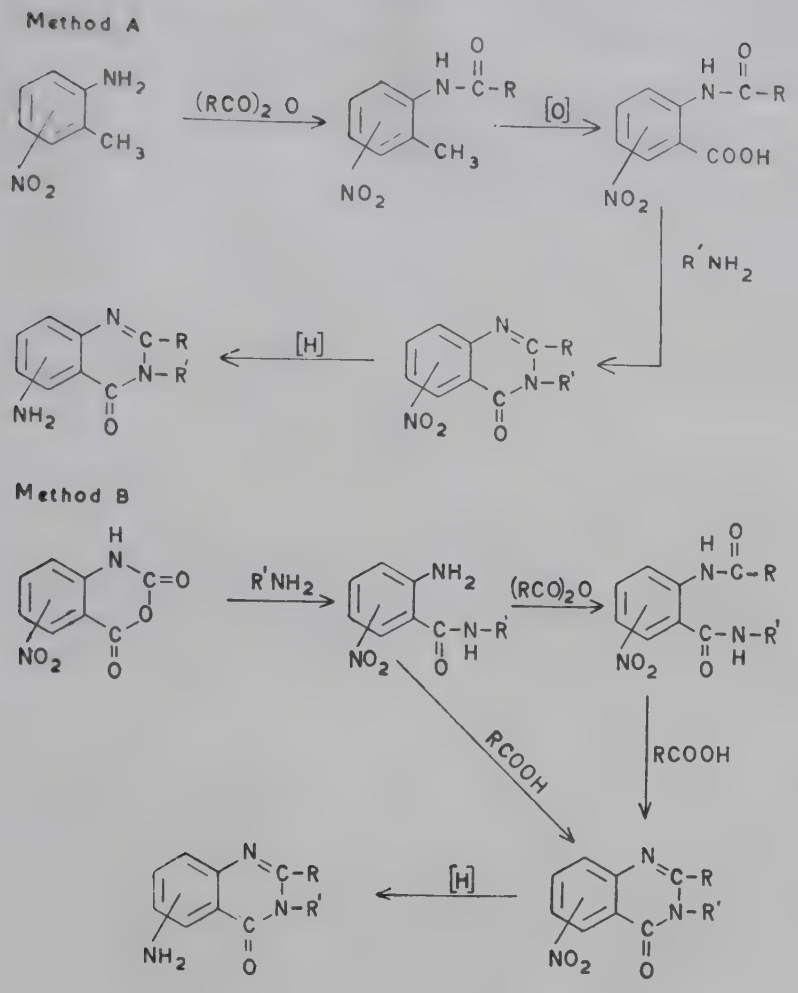


FIG. 1

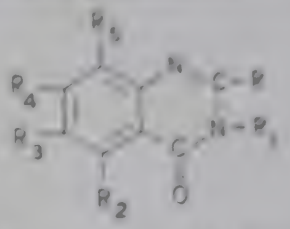
USA. Infrared absorption spectra were recorded on a Perkin-Elmer (Model 221) spectrophotometer.

As illustrations of the two synthetic routes which were used in the preparation of the amino substituted quinazolinones, the preparation of 2-methyl-3-(*o*-tolyl)-6-amino-4 (3*H*)-quinazolinone (III) and 2-ethyl-3-(*o*-tolyl)-6-amino-4 (3*H*)-quinazolinone (IV) will be offered in detail representing methods A and B, respectively.

The infrared absorption spectra of the compounds listed in Table 1 were examined. The spectra for compounds (III) and (IV) showed distinct splitting in the region of 3500–3300$^{cm.-1}$ consistent with characteristic bands for primary amines. Quinazolinone identifying bands at 1628–1618$^{cm.-1}$, 1581–1566$^{cm.-1}$ and 1517–1478$^{cm.-1}$ were present.

Compound III (Method A)

***N*-Acetyl-2-methyl-4-nitroaniline.** Fifty ml. of acetic anhydride and 30.5 g. (0.20 mole) of 2-methyl-4-nitroaniline were mixed and refluxed



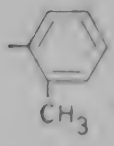
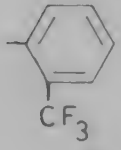
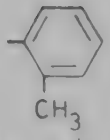
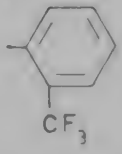
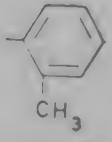
Compd.	R	R_1	R_2	R_4	R_5	R_3	Method	M.P.
I	$-CH_3$		NH_2	H	H	H	B	
II	H	do	H	$-NH_2$	H	H	A B	
III	$-CH_3$	do	H	$-NH_2$	H	H	A	
IV	$-CH_2CH_3$	do	H	$-NH_2$	H	H	A B	
V	$-CH_2CH_2CH_3$	do	H	$-NH_2$	H	H	A	
VI	$-CH(CH_3)_2$	do	H	$-NH_2$	H	H	A	
VII		do	H	$-NH_2$	H	H	A B	
VIII		do	H	$-NH_2$	H	H	A B	
IX	$-CH_3$	do	H	-NHCHO	H	H	A	
X	$-CF_3$	do	H	$-NH_2$	H	H	A	
XI	H		H	$-NH_2$	H	H	A	
XII	$-CH_3$	do	H	$-NH_2$	H	H	A	
XIII	$-CH_3$	do	H	-NHCHO	H	H	A	
XIV	$-CH_3$		H	H	$-NH_2$	H	A	
XV	$-CH_2CH_2CH_3$	do	H	H	$-NH_2$	H	A	
XVI	$-CH_3$		H	H	$-NH_2$	H	A	
XVII	$-CH_3$		H	H	-NHCHO	H	A	
XVIII	$-CH_3$	do	H	H	H	$-NH_2$	A	

TABLE 1

RMULA	Yield, %	Carbon, %		Hydrogen, %		Nitrogen, %		Fluorine, %	
		Calc.	Found	Calc.	Found	Calc.	Found	Calc.	Found
$H_{15}N_3O$	56	72·43	72·18	5·70	5·83	15·84	15·96	..	..
$H_{13}N_3O$	65 80	71·69	71·52	5·21	5·13	16·72	16·76	..	..
$H_{15}N_3O$	33	72·43	72·35	5·70	5·65	15·84	15·93	..	..
$H_{17}N_3O$	25 78	73·09	72·85	6·13	6·17	15·04	15·20	..	..
$H_{19}N_3O$	29	73·69	73·70	6·53	6·49	14·33	14·27	..	..
$H_{19}N_3O$	41	73·69	73·80	6·53	6·69	14·33	14·15	..	..
$H_{17}N_3O.\ H_2O$	20 40	69·88	69·96	6·19	6·33	13·58	13·61	..	..
$H_{19}N_3O$	24 48	74·73	74·93	6·27	6·47	13·76	13·74	..	..
$H_{15}N_3O_2$	29	69·61	69·77	5·15	5·33	14·33	14·47	..	..
$H_{12}F_3N_3O$	37	60·19	60·44	3·79	4·11	13·16	13·21	17·85	17·58
$H_{10}F_3N_3O$	40	59·02	59·05	3·30	3·27	13·76	13·70	18·67	18·71
$H_{12}F_3N_3O$	36	60·19	60·38	3·79	4·02	13·16	13·29	17·85	17·71
$H_{12}F_3N_3O_2$	32	59·79	59·82	3·48	3·49	12·10	12·49	16·41	16·20
$H_{15}N_3O$	24	72·43	72·14	5·70	5·69	15·84	15·93	..	..
$H_{19}N_3O$	46	73·69	73·63	6·53	6·64	14·33	14·40	..	..
$H_{12}F_3N_3O$	40	60·19	59·88	3·79	4·00	13·16	12·98	17·85	18·01
$H_{15}N_3O_2$	23	69·61	69·42	5·15	5·50	14·33	13·90	..	..
$H_{15}N_3O$	40	72·43	72·12	5·70	5·67	15·84	15·79	..	..

for 30 min. The solution was cooled below 40°C. and poured with stirring into 500 ml. of ether while cooling in an ice bath. The solid was filtered off and the filtrate was concentrated to dryness to give a few more grams of the solid product. The combined solid was dissolved by heating in 300 ml. of methyl ethyl ketone, decolorized with 20 gm. of animal charcoal, filtered through filter-cel, concentrated *in vacuo* to about 50 ml. and then added to 2 litres of petroleum ether. The resulting solid, *N*-acetyl-2-methyl-4-nitroaniline was filtered off and air-dried: yield 32·8 g. (85·0%); m.p. 196–97°C., (lit.[5], m.p. 198–200°C.).

***N*-Acetyl-5-nitroanthranilic acid.** Fifty grams (0·26 mole) of *N*-acetyl-2-methyl-4-nitroaniline, 60·0 g. of magnesium sulphate heptahydrate and 3 litres of water were added to a 5-litre, 3-necked, round-bottomed flask equipped with a stirrer, a condenser and a thermometer. The reaction mixture was heated to 80°C. and with stirring 150·0 g. of potassium permanganate was added portionwise during 3 hr. maintaining the temperature at 80–85°C. The stirring and heating was continued at 80°C. for an additional 3 hr. It was filtered hot and the filtrate acidified with concentrated hydrochloric acid. The resulting solid, *N*-acetyl-5-nitroanthranilic acid, was removed by filtration, washed with water and air-dried: yield 38·0 g. (67%); m.p. 218–19°C. (lit.[6], m.p. 212–14°C)..

2-Methyl-3-(*o*-tolyl)-6-nitro-4(3*H*)-quinazolinone. Thirty grams (0·134 mole) of *N*-acetyl-5-nitroanthranilic acid, 21·0 g. (0·20 mole) of *o*-toluidine and one litre of toluene were placed in a 3-litre, 3-necked, round-bottomed flask provided with a stirrer, a condenser and a dropping funnel. With stirring, a solution of 15·0 g. (0·10 mole) of phosphorus trichloride in 100 ml. of toluene was added dropwise during a one hour period. The reaction mixture was refluxed for 2·5 hr, cooled to below 30°C. and neutralized with 10% sodium carbonate solution. The toluene layer was separated and the aqueous layer was extracted with 200 ml. of toluene. The combined extract was subjected to steam distillation, the resulting mixture was cooled and the solid, 2-methyl-3-(*o*-tolyl)-6-nitro-4(3*H*)-quinazolinone was removed by filtration, air-dried and recrystallized three times from 300 ml. each of absolute methanol: yield 30·0 g. (76·0%); m.p. 175–77°C. (lit.[7], m.p. 175–77°C.).

2-Methyl-3-(*o*-tolyl)-6-amino-4(3*H*)-quinazolinone (III). Thirty grams (0·10 mole) of 2-methyl-3-(*o*-tolyl)-6-nitro-4(3*H*)-quinazolinone was suspended in 280 ml. absolute methanol and hydrogenated at 65 p.s.i. using 16·0 g. Raney nickel catalyst. Hydrogenation was completed in one hour. The reduction mixture was warmed, filtered hot, and the filtrate concentrated to dryness *in vacuo*. The near-white solid product was stirred with 50 ml. of methanol, and filtered off. It was recrystallized from 300 ml. of absolute methanol: yield 23·1 g. (86·5%); m.p. 214–15°C. (lit.[7], m.p. 214–15°C.).

2-Methyl-3-(*o*-tolyl)-6-formamido-4 (3*H*)-quinazolinone (IX). Acetic formic anhydride[8] was prepared by heating 20·4 ml. acetic anhydride and 8·8 ml. formic acid (98%) for 2 hr at 50–60 °C. in a flask equipped with a stirrer, a condenser and a drying tube. The solution was cooled to 0 °C. at which temperature 40·0 g. (0·15 mole) of 2-methyl-3-(*o*-tolyl)-6-amino-4 (3*H*)-quinazolinone (III) was added followed by 50 ml. of absolute ether. The mixture was stirred for 26 hr and the resulting solid product (IX) was filtered off and dried. It was recrystallized twice from absolute methanol: yield 42·0 g. (96%); m.p. 229–30°C.

Compound IV (Method B)

N-(*o*-**tolyl**)-**2-amino-5-nitrobenzamide.** Forty grams (0·192 mole) of 6-nitroisotoic anhydride and 53 g. (0·50 mole) of *o*-toluidine were thoroughly mixed in a beaker and then warmed slowly to a melt at which temperature there was much evolution of gas (carbon dioxide), after which the mixture solidified. The solid was recrystallized from 1600 ml. of 95% ethanol: yield 38·5 g. (74·0%); m.p. 210–12°C. Anal. calc. for $C_{14}H_{13}N_3O_3$: N, 15·48; found: N, 15·40.

N-(*o*-**tolyl**)-**2-propionamido-5-nitrobenzamide.** Nine grams (0·033 mole) of *N*-(*o*-tolyl)-2-amino-5-nitrobenzamide, 8·5 g. (0·065 mole) of propionic anhydride and 150 ml. ethyl acetate were mixed together and warmed on a steam bath until solution occurred. The ethyl acetate was allowed to boil off, after which time the reaction mixture solidified. The light yellow solid was washed several times with ether and air-dried: yield 10·2 g. (93%); m.p. 184–85°C. Anal. calc. for $C_{17}H_{17}N_3O_4$: N, 12·84; found: N, 12·90.

2-Ethyl-3-(*o*-tolyl)-6-nitro-4 (3*H*)-quinazolinone. Ten grams (0·03 mole) of *N*-(*o*-tolyl)-2-propionamido-5-nitrobenzamide and 4·8 ml. (0·064 mole) of propionic acid were mixed together in a 25 ml. round-bottomed flask, warmed to gentle reflux for 6 hr and poured into 400 ml. water. The solid was filtered off and recrystallized from 95% ethanol: yield 7·4 g. (78%); m.p. 124–26°C. Comparison of this product with that obtained by the condensation of *N*-propionyl-5-nitroanthranilic acid and *o*-toluidine showed the two intermediates to be the same, by mixed melting points and thin layer chromatography.

2-Ethyl-3-(*o*-tolyl)-6-amino-4 (3*H*)-quinazolinone (IX). Seven and one-half grams (0·024 mole) of 2-ethyl-3-(*o*-tolyl)-6-nitro-4 (3*H*)-quinazolinone was dissolved in 220 ml. absolute methanol and hydrogenated at 60 p.s.i. using 3·5 g. Raney nickel catalyst. Reduction was completed within 2 hr. The reaction mixture was warmed, filtered and then concentrated to a semi-solid. Recrystallization from 50% ethanol gave a solid after long standing. It was recrystallized again from 150 ml. isopropyl alcohol: yield, 4·8 g. (71%); m.p. 158–60°C.

PHARMACOLOGY

The anticonvulsant and general pharmacodynamic properties of 2-methyl-3-(*o*-tolyl)-4(3*H*)-quinazolinone (Tuazole®)* have been described by Swift *et al.*[9]. As a result of their investigations these authors concluded:

(1) In the intact animal, Tuazole was a relatively neurotoxic substance, but in high doses, produced marked neurological changes and cholinergic effects. The most pronounced of these was a flaccid paralysis.

(2) In mice, Tuazole elevated the threshold of electrically induced seizures and potentiated the hypnotic effect of hexobarbital. It was the most potent antagonist of strychnine tested. It also antagonized the tremor but intensified the autonomic effects of tremorine.

(3) Direct studies in cats have shown that Tuazole depressed polysynaptic reflex arcs in doses which did not inhibit monosynaptic reflexes, spinal ganglia, the myoneural junction or skeletal muscle.

(4) Tuazole relaxed smooth muscle directly and had a weak antihistaminic effect. No analgesic or diuretic activity was present.

(5) Since the principal effects elucidated were central in origin, the pharmacology of Tuazole can be discussed in terms of spinal depression and a shift in autonomic balance toward cholinergic predominance.

As part of a continuing research programme, over one hundred substituted 4-(3*H*)-quinazolinones have been submitted to the Pharmacology Department, Strasenburgh Laboratories, by the Organic Chemistry Department.

These compounds represent various types of deviation from the Tuazole structure.

The pharmacologic screening programme is designed to reveal basic central nervous system activity. The tests used in this phase of evaluation are as follows:

1. *The neurological deficit test*—This is our adaptation of the Dunham and Miya rota-roller test. It is not stressed as a major component of our screen. The test is used as a preliminary trial run to obtain dose ranges for subsequent electroshock and strychnine antagonism test and to determine the time of peak effect.

2. *Electroshock seizure threshold determination*—Our modification of this standard technique enables us to determine with statistical precision the actual electroshock threshold of the mouse population in quasi-pharmacological terms (i.e. mA/gm.). Further, the actual increase or decrease of this measure in response to a drug can be determined in contrast with the standard procedure which is a quantal type of test based on the ability of a drug to affect a 20 per cent elevation of threshold current.

3. *Maximal electroshock seizure test*—This is essentially another threshold test in our hands. The 'current-dose' principle is used to determine the

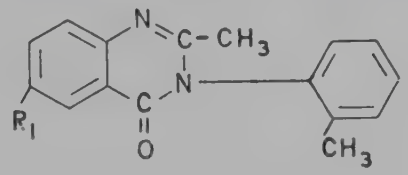
*Tuazole® is the Strasenburgh Laboratories' brand of Methaqualone.

parameter for producing an opisthotonic seizure in most mice (i.e. maximal convulsant dose or CD_{99}). The test then determines the drug dose which will protect 50 per cent of the animals upon decreasing their sensitivity to the current.

4. *Strychnine antagonism test*—We have made little or no modification of the standard published procedure for this test. The convulsant dose (CD_{99}) of strychnine has been determined to be 2·5 mg./kg. by the intraperitoneal route in our mice. Some investigators use lower doses but we suspect that this is a mouse strain difference. Other tests routinely carried out are: (a) hexobarbital potentiation; (b) tremorine antagonism, and (c) analgesic evaluation—hot plate and *p*-phenylquinone.

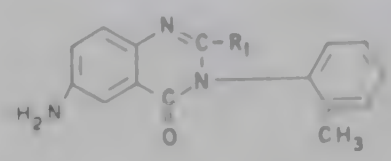
Data collected from these screening procedures are used to determine whether or not investigative compounds will be subjected to additional comprehensive pharmacological evaluation. Since this presentation is intended to describe only the analgetic properties of certain amino-substituted quinazolinones, I shall restrict the presentation to data obtained from evaluation in this area. The only deviation from this theme will be the presentation of some acute toxicity data which is pertinent to the development of structure-activity relationships. Tables 2, 3 and 4 deal specifically with the influence of substitution on acute toxicity and analgesic

TABLE 2—BIOLOGICAL ACTIVITIES OF 6-SUBSTITUTED 2-METHYL-3-(*o*-TOLYL)-4-QUINAZOLINONES



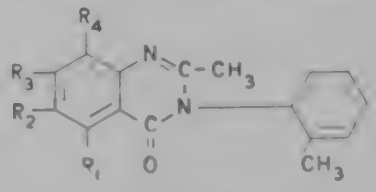
R_1	Approximate LD_{50} (mouse) *mg./kg. (oral)*	Analgesic activity $\leqslant$20 *mg./kg. (oral)*
H	300	Absent
—Cl	>500	Absent
—Br	>500	Absent
—NO_2	250	Absent
—NH_2	100	Significant
—CH_3	>500	Absent
—OCH_3	>500	Absent
—OH	>500	Absent
—NHCHO	125	Absent

TABLE 3—EFFECT OF SUBSTITUTION AT 2-POSITION ON BIOLOGICAL ACTIVITIES OF 3-(*o*-TOLYL)-6-AMINO-4-QUINAZOLINONES



R_1	Approximate LD_{50} (mouse) *mg./kg. (oral)*	Analgesic activity <20 *mg. kg. (oral*
H	104	Present
$-CH_3$	100	Significant
$-CH_2CH_3$	147	Present
$-CH_2CH_2CH_3$	109	Significant
$-CH(CH_3)_2$	262	Absent
$-CF_3$	>500	Absent

TABLE 4—BIOLOGICAL ACTIVITIES OF AMINO SUBSTITUTED 2-METHYL-3-(*o*-TOLYL)-4-QUINAZOLINONES



Position of $-NH_2$	Approximate LD_{50} (mouse) *mg./kg. (oral)*	Analgesic activity <20 *mg./kg. (oral)*
R_1	77	Absent
R_2	100	Significant
R_3	>500	Present
R_4	>500	Absent

activity. The analgesic data were obtained using the hot-plate test. Groups of ten mice were administered the test compound orally in doses ranging to 20 mg./kg. The designations *present* and *significant* have the following meaning. The term present means that analgesic activity was demonstrated only by one or two animals in a group of ten. The term significant described a 50 per cent or greater response in a group of ten treated animals. Table 2 contains data describing the effect of various substituent groups in the 6-position of the aryl ring of the quinazolinone structure. These data indicate

the most significant findings with respect to both toxicity and analgesic activity, when the 2-methyl-3-(*o*-tolyl) (6-substituted-4-quinazolinone) has an amino group in the designated R_1 position. It also indicates the low dose requirement for acute toxicity of the formamido compound. In addition, this compound demonstrated no analgesic activity.

From the data in Table 3 the effects of varying the substitution in the 2-position of several 2-substituted 3-(*o*-tolyl)-6-amino-4-quinazolinones are noticeable. There appears to be a correlation between the length of the alkyl chain, the dose required for acute lethality and the degree of analgesic activity demonstrated by the compound.

In Table 4, we have summarized a similar set of data collected on compounds in which the amino group was rotated throughout four positions of the aryl ring. It may be noted here that the most significant analgesic activity was demonstrated by the compound in which the amino group was located at position R_2. The corresponding decrease in acute toxicity as analgesic activity disappeared, is also noticeable.

With these data we felt it necessary to subject compounds having a 6-amino group and alkyl substitutions of varying length in the 2-position to more comprehensive analgesic testing. For this purpose, the pressalgesic test described by Randall and Selitto was selected.

METHOD

General Procedure

Manor Farm rats of either sex and weighing from 83 to 125 g. were used in this study. The rats were separated into treated groups of five each per dosage level of compound administered. A non-treated group of five rats were simultaneously utilized with each test. All of the animals were fasted for 16 hr prior to use.

On the day of the test two threshold pressure values obtained one hour apart (recorded in mm. /Hg) were recorded from both hind paws of each rat by a modified method originally described by Randall and Selitto[10]. The instrument utilized in these experiments was activated by compressed nitrogen which was regulated by two-foot operated electrically controlled pneumatic air valves. One valve controlled the compressed air entering a system which terminated in an inverted 10 ml. syringe. A blunt metal point was affixed to the barrel of the syringe. The syringe was clamped in a perpendicular plane to a heavy ring stand in such a manner that when air entered the system the barrel was driven toward the table surface. The rat's foot was held manually in a specially constructed metal trough directly under the syringe barrel so that the metal point would impinge upon the plantar surface of the foot. The pressure in the system was recorded by an anemometer interposed into the system with a T tube. The end-point used was vocalization and/or struggle and biting by the animal at the peg impinging upon the foot. The air-flow into the system was main-

lated constant at 20 mm Hg per sec. When the end-point was reached, the foot pedal was released which automatically stopped inflow air into the system and held the pressure constant at end-point. This enabled the operator to read the mm.Hg pressure without error. A second foot pedal activating an electrically operated pneumatic valve released air pressure after the reading had been made.

After the two initial control valves had been obtained for each rat, the right hind paw of each animal was 'sensitized' or inflamed by a subcutaneous injection into the midplantar surface of 0·1 ml. of a 20 per cent suspension of Brewer's yeast in physiological saline solution. Control threshold pressure values were again obtained on both the inflamed and non-inflamed hind paw of each animal at one and two hours after the yeast injection. The value obtained at two hours was used as the pre-drug control value.

The threshold values with standard deviations for the non-inflamed and the inflamed foot of the 20 control rats are shown in Fig. 2. It can be seen that the standard deviations for the inflamed foot values are much smaller than those obtained from the non-inflamed foot. Thus, elevation

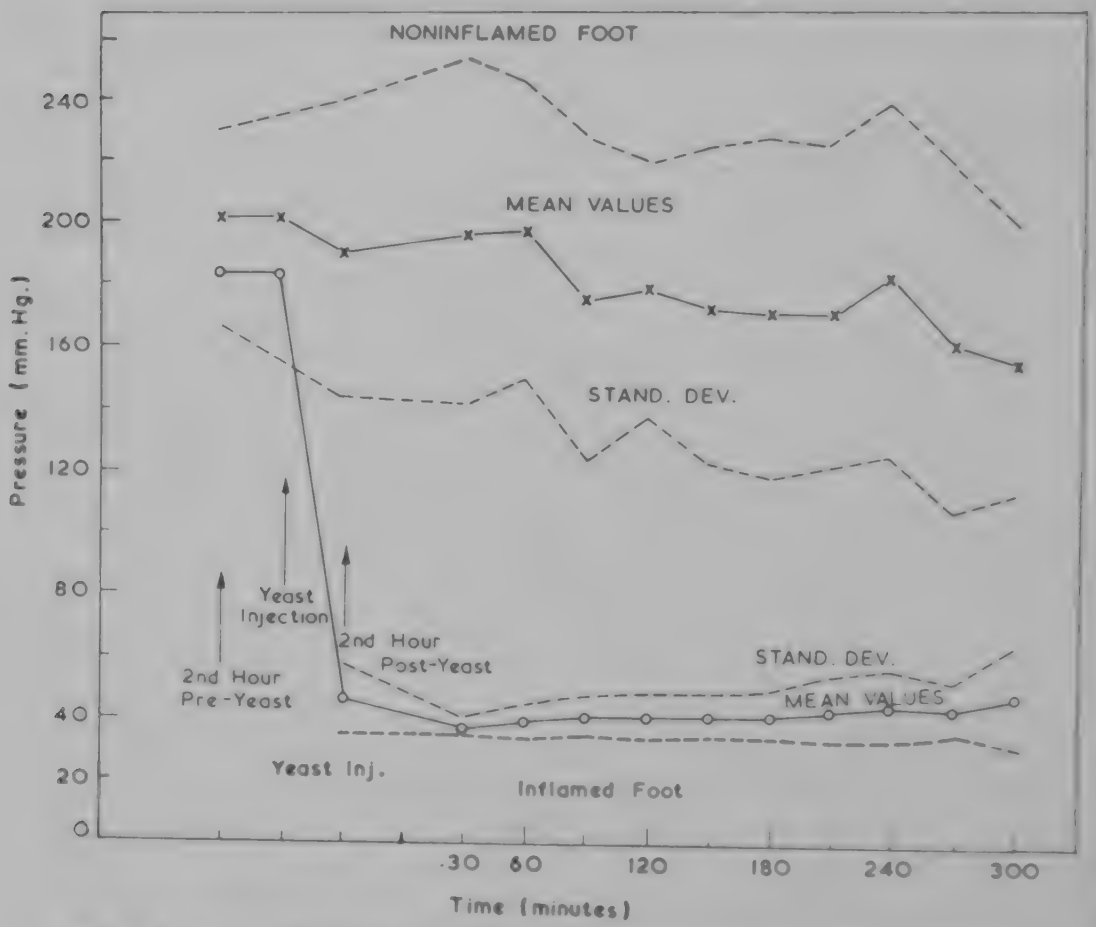


FIG. 2.—CONTROL THRESHOLD VALUES AND STANDARD DEVIATIONS OBTAINED FROM NONTREATED RATS

in threshold of the inflamed foot by non-narcotic analgetic substances into the area of statistical significance is much more easily detected than measurements obtained from the non-inflamed foot. However, potent morphine-like compounds produce significant elevations in threshold of both feet. Non-narcotic-type analgesics occasionally may elevate the threshold of the non-inflamed foot into the area of statistical significance.

Dose-Response Curves

Each compound tested was administered orally at dosage levels of 5, 10 and 20 mg./kg. to groups of five rats per dosage level. A control group of five rats was administered the vehicle only.

The compounds were suspended in an aqueous dispersion of 4 per cent cleargel in such a manner that volumes administered to each rat could be maintained constant at 1 ml./100 g. of body weight.

All analgesic trials were blind studies. Not only did the operator not know which group of animals had received the test of control substances, but he also did not know the dosage levels administered to a given group. Furthermore, a helper handed animals to the operator in random fashion so that no entire group at any dosage level could be evaluated in a consecutive manner. An attempt was made to remove all bias from the test by this means.

Pressalgesic values of both hind paws of each rat were obtained at 30, 60, 90, 120, 150, 180 and 240 min. after compound administration. If activity was absent at 120 min. or still present at a significant level at 240 min. the study was terminated or continued as dictated by the results at that time.

RESULTS

Analgesia

At least two compounds showed an analgesic response of significance, producing a peak effect at 60 to 90 min. after dosing, dependent upon the dosage level. 2-(*n*-Propyl)-3-(*o*-tolyl)-6-amino-4 (3*H*)-quinazolinone demonstrated a significant analgesic response at all dosages employed (Fig. 3). Peak analgesic effect with this compound occurred at 60 min. after administration at the 5 and 10 mg./kg. dosage levels. At the 20 mg./kg. dosage level, peak activity occurred at 90 min. after administration. Analgesia was still present at all dosage levels at the five-hour determination. Tests were not carried beyond the five-hour reading.

The threshold values for the non-inflamed foot of the rats used were also increased. Even though the standard deviation of the values obtained from the control rats was great, the values obtained from the rats administered this compound were still significant at the 95 per cent level of confidence (greater than two standard deviations from the mean of the control

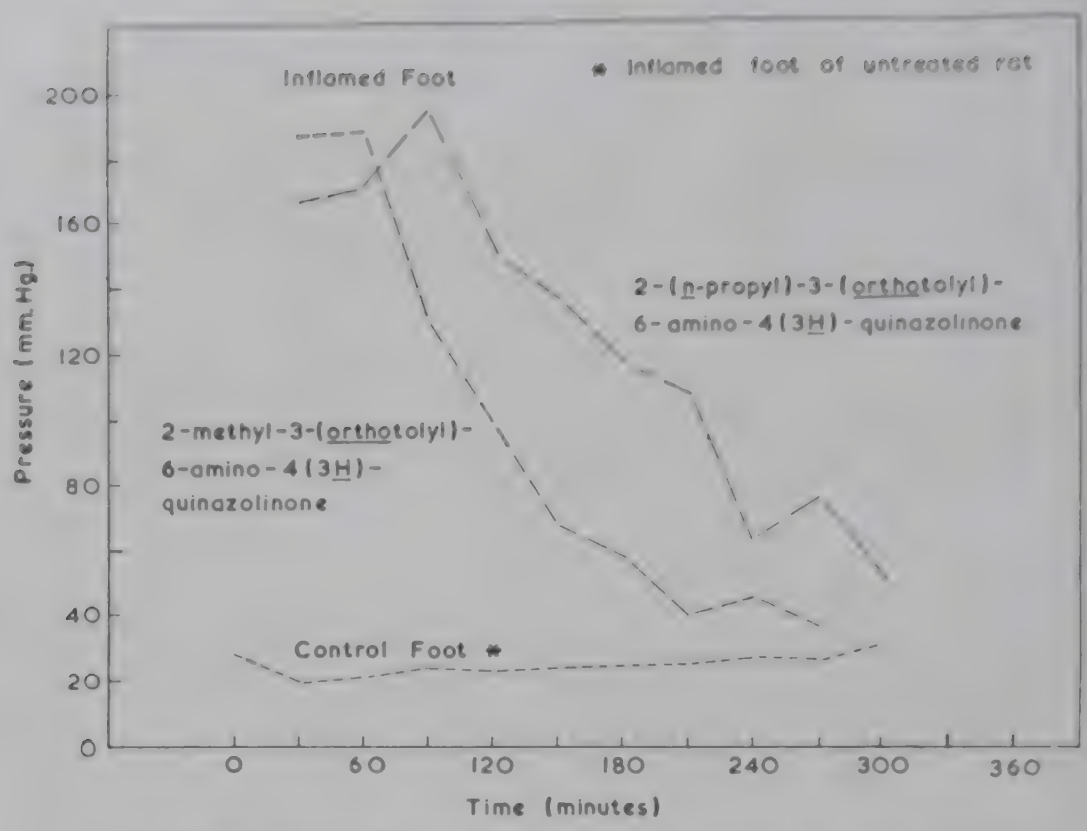


FIG. 3 — TIME DURATION CURVES OF THE ANALGESIC ACTIVITY OF TWO 6-AMINO SUBSTITUTED QUINAZOLINONES

group). These results would indicate that the analgesic activity of the compound was not due to an anti-inflammatory mechanism.

2-Methyl-3-(*o*-tolyl)-6-amino-4(3*H*)-quinazolinone did not produce an analgesic effect at dosage below 20 mg./kg. However, at 20 mg./kg., highly significant values were obtained. Analgesic activity appeared at peak one hour after administration and continued for at least three hours.

Pharmacodynamics

2-Methyl-3-*o*-tolyl-6-amino-4(3*H*)-quinazolinone. Slight ataxia, sedation, flaccidity and sluggish righting reflexes were observed from 30 to 90 min. particularly at the upper dosage level of 20 mg./kg. All of the groups were quieted and sedated through three hours.

2-*n*-Propyl-3-*o*-tolyl-6-amino-4(3*H*)-quinazolinone. At 30 min. animals at all dosage levels appeared moderately sedated and quieted. Hypoactivity and bradypnea were apparent. At 60 min., the above signs were more pronounced and flaccidity, ataxia and reduced righting reflexes were exhibited, particularly at 10 and 20 mg./kg. dosage levels. One animal at 20 mg./kg. was near prostration. At two hours, the above signs were still present. Two animals-of-five at 20 mg./kg. were near prostration. All signs began to diminish at three hours. At five hours, slight sedation was still apparent, particularly at the upper dosage level of 20 mg./kg.

DISCUSSION

Although the rats administered the two compounds under investigation exhibited considerable flaccidity and reduced reflexes, these were still able to react to pain stimuli by vocalization and struggle. Thus the analgesic effects noted were considered not due to the inability of the animal to respond to the pressalgesic stimulus to the hind feet. Furthermore, the analgesic activity continued beyond the time when the animals had regained full coordination of body movement. Neither was the analgesic activity observed considered to be due to an anti-inflammatory effect of the agents, since the non-inflamed foot threshold valves were also elevated.

Butazolidin, a well known nonsteroidal anti-inflammatory agent has been shown to elevate the analgesic threshold values of the inflamed feet of rats without altering the inflammatory response of the same feet as determined by measurement of the foot volume. Furthermore, steroidal compounds (Cortisone, hydrocortisone and dexamethansone) have been shown to be ineffective in elevating the pain threshold of inflamed feet at dosages which produce an anti-inflammatory response as determined by the carrageeninpouch technique.

In view of these data, it is concluded that the analgesic responses observed were realistic and meaningful. In comparison with dose response curves previously obtained with aspirin in the identical test procedure, 2-(*n*-propyl)-3-(*o*-tolyl)-6-amino-4 (3*H*)-quinazolinone is 40 times as potent and of similar duration. However, the dosages at which aspirin demonstrates significant analgesic (200 to 400 mg./kg.), pharmacodynamic signs are rarely observed.

REFERENCES

1. Kacker, I. K. & Zaheer, S. H., *J. Indian chem. Soc.*, **28** (1951), 344.
2. Gujral, M. L., Saxena, P. N. & Tiwari, R. S., *Indian J. med. Res.*, **43** (1955), 637.
3. Grimmel, H. W., Guenther, A. & Morgan, J. F., *J. Am. chem. Soc.*, **68** (1946), 542.
4. Jackman, G. B., Petrow, V. & Stephenson, O., *J. Pharm. Pharmac.*, **12** (1960), 529; Brit., 978,356.
5. Dunn, G. E. & Prysiazniuck, R. Y., *Canad. J. Chem.*, **39** (1961), 285.
6. Berkenheim, A. M. & Livshits, R. S., *J. Gen. Chem.*, (U.S.S.R.), **6** (1936), 1025.
7. Brit. 916,139.
8. Huffman, C. W., *J. org. Chem.*, **23** (1958), 727.
9. Swift, John G., Dickens, Elsie A. & Becker, Bernard A., *Arch. int. Pharmacodyn.* **CXXVIII** (1-2), 1960.
10. Randall & Selitto, *Arch. int. Pharmacodyn.*, **111** (1957), 409.

2-Alkyl-3-Aralkyl-4 (3*H*) Quinazolones as CNS Depressants

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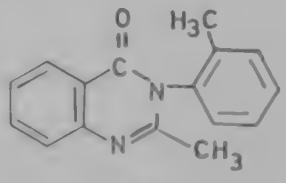
Introduction

4 (3*H*) quinazolones have been investigated for a variety of physiological effects such as hypnotic[1], analgesic[2], antitussive[3], anticholinergic action[4], anti-spasmodic[5], muscle relaxant[6] and anti-inflammatory properties[7].

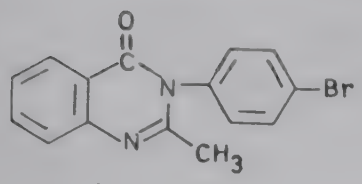
The work of Kacker and Zaheer[8] gave an impetus to development of the chemistry of 4 (3*H*) quinazolones which had led to the introduction of 2-methyl-3-*o*-tolyl-4 (3*H*) quinazolone (I) into therapy as a non-barbiturate hypnotic. Clinical efficacy of 2-methyl-3-(*o*-tolyl)-4 (3*H*) quinazolone has been reported by Duchastel[9] who carried out a double blind study on 100 patients. He observed that 87 cases had good hypnotic effectiveness without adverse side effects. However, in anxiety cases, in less than hypnotic doses, it had no effect as a tranquillizer.



(I)

The presence of chlorine and bromine in *ortho* and *para* positions in the aryl nucleus at the 3 position has shown promising activity.

2-Methyl-3-(*p*-bromophenyl)-4 (3*H*) quinazolone (II) has been claimed as an effective anticonvulsant in electroshock induced mice[10]. 2-Methyl-3-(*o*-chlorophenyl)-4 (3*H*) quinazolone (III) has been claimed as a hypnotic[11].



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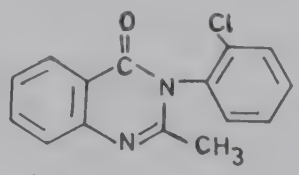
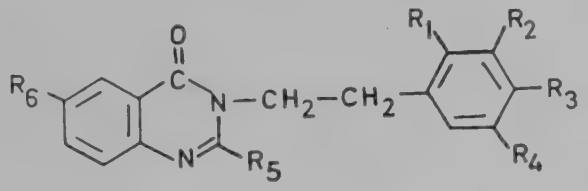


(III)

It is evident from the existing literature that the chemistry and pharmacology of 4 (3*H*) quinazolones have been exhaustively studied with an aryl group at 3 position. We thought it of interest to put an aralkyl group at the 3 position of the quinazolone nucleus and the present work deals with the preparation and physiological properties of 4 (3*H*) quinazolones (IV) having a variety of such substituents at 3 position.



(IV)

CHEMISTRY

The various 4 (3*H*) quinazolones discussed in this work were prepared by the reaction of appropriately substituted β-phenylethylamines with suitable *N*-alkanoylanthranilic acids.

Starting Materials and Intermediates

β-Phenylethylamine was obtained from Aldrich Chemicals, USA; 2-, 3-, and 4-chloro-; 2-, and 4-methoxy-; 2, 3- and 3, 4-dimethoxy-; 3-methoxy-4-ethoxy-; 4-methyl- and 3, 4-dichlorophenylethylamines were prepared as described in literature. 2, 3-Dichloro-, 2-methyl-3-chloro, 2-methyl-5-chloro- and 2-chloro-5-methyl phenylethylamines are new compounds and were synthesized by the following route:

Aldehyde → Cinnamic acid → Propionic acid → β-Phenylethylamine

The aldehydes were obtained by Beech reaction[12] of the corresponding anilines. Condensation with malonic acid yielded the suitably substituted cinnamic acids which, on reduction with sodium amalgam, gave the corresponding propionic acids. The latter afforded the β-phenylethylamines by the Schmidt reaction, according to the following general procedure.

To a mixture of the β-phenylpropionic acid (25 g.), benzene (150 ml.) and sulphuric acid (120 ml.) was added sodium azide (12·5 g.) in small portions with stirring. The reaction mixture was then heated at 60° for 2 hr and poured on crushed ice. Basification with 20 per cent sodium hydroxide solution and extraction with ether afforded the crude amine which was distilled under reduced pressure.

Table 1 gives the preparative and analytical data for the intermediates and the phenylethylamines.

4 (3*H*) Quinazolones. These were prepared (i) according to the procedure of Grimmel *et al.*[14] or (ii) using phosphorus oxychloride as a condensing agent as described below.

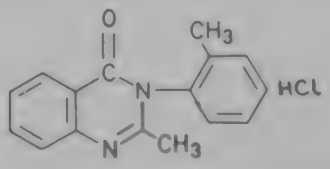
A mixture of the β-phenylethylamine (0·1 M), *N*-alkanoyl anthranilic acid (0·1 M) and phosphorus oxychloride (35 ml.) was refluxed for 2 hr. The reaction mixture was then poured over crushed ice and made alkaline with 20 per cent caustic soda solution. The solid product was filtered and washed with water.

The various 4 (3*H*) quinazolones synthesized and their analytical data are presented in Table 2.

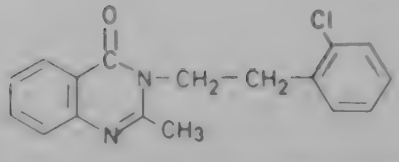
PHARMACOLOGY

Some of the quinazolones discussed above were screened for CNS activity, at Riker Laboratories, California. Further work on these compounds was done by the Pharmacological Research Unit of CSIR, at Department of Pharmacology, Seth G. S. Medical College, Bombay. In general, these compounds, exhibited sedation, and tranquillizing activity. Their hypnotic property was weaker than methaqualone and they differed from methaqualone in not possessing anticonvulsant property. Two of the compounds of the series, designated as R G-5 2 and R G-5 5, were taken up for further studies (Fig. 1).

During primary screening in mice, these compounds produced sedation, loss of righting reflex, relaxation of muscle tone, and ptosis. These effects were comparable to that of methaqualone. Compounds of the present series produced sedation, at a dose which did not produce ataxia. None of the

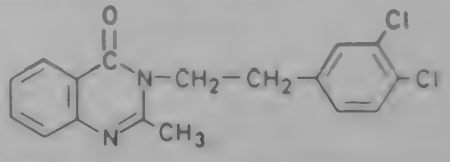


2-METHYL-3-O-TOLYL-4(H) QUINAZOLONE
(METHAQUALONE HYDROCHLORIDE



2-METHYL-3-(2-CHLOROPHENYLETHYL)
-4(3H)-QUINAZOLONE

RG 5/2



2-METHYL-3(3'-4'-DICHLOROPHENYLETHYL)
-4(3H)-QUINAZOLONE

RG 5/5

FIG. 1

TABLE 1—PREPARATIVE AND ANALYTICAL DATA FOR INTERMEDIATES

	M.P. °C	B.P. °C (*mm.*)	Yield %	Mol. formula	Analysis, % Found C	Found H	Found N	Calc. C	Calc. H	Calc. N
Benzaldehydes										
2, 3-Dichloro-	..	108-110 (3-4)	40	$C_7H_4Cl_2O$	47·86	2·36	..	47·94	2·30	..
...Semicarbazone	238-239	..	..	$C_8H_7Cl_2N_3O$	..	..	18·34	..	..	18·10
2-Methyl-3-chloro-	..	128-132(6)	44	C_8H_7ClO	61·94	4·38	..	62·16	4·56	..
...Semicarbazone	220	..	..	$C_9H_{10}ClN_3O$	..	..	19·76	..	..	19·85
2-Methyl-5-chloro-	..	98-102(1·5)	46	C_8H_7ClO	62·33	4·46	..	62·16	4·56	..
...Semicarbazone	239-240	..	..	$C_9H_{10}ClN_3O$	..	..	19·98	..	..	19·85
2-Chloro-5-methyl-	..	90-94(1·5)	44	C_8H_7ClO	62·42	4·62	..	62·16	4·56	..
...Semicarbazone	242	..	..	$C_9H_{10}ClN_3O$	..	..	19·81	..	..	19·85
Cinnamic acids										
2, 3-Dichloro-	228	..	..	$C_9H_6Cl_2O_2$	49·68	2·86	..	49·79	2·79	..
2-Methyl-3-chloro-	196	..	..	$C_{10}H_9O_2Cl$	61·38	4·56	..	61·08	4·62	..
2-Methyl-5-chloro-	184	..	..	$C_{10}H_9O_2Cl$	61·22	4·48	..	61·08	4·62	..
2-Chloro-5-methyl-	132	..	..	$C_{10}H_9O_2Cl$	61·02	4·76	..	61·08	4·62	..
Propionic acids										
β-2, 3-Dichlorophenyl-	114	..	..	$C_9H_8Cl_2O_2$	49·40	3·72	..	49·33	3·68	..
β-2-Methyl-3-chlorophenyl-	110	..	..	$C_{10}H_{11}O_2Cl$	60·62	5·48	..	60·46	5·58	..
β-2-Methyl-5-chlorophenyl-	74	..	..	$C_{10}H_{11}O_2Cl$	60·36	5·52	..	60·46	5·58	..
β-2-Chloro-5-methylphenyl-	54	..	..	$C_{10}H_{11}O_2Cl$	60·58	5·44	..	60·46	5·58	..
β-Phenylethylamines										
2, 3-Dichloro-	..	130-132(5-6)	..	$C_8H_9Cl_2N$	50·64	4·86	7·44	50·55	4·77	7·37
2-Methyl-3-chloro-	..	110-116(4)	..	$C_9H_{12}ClN$	64·23	7·36	8·38	64·06	7·13	8·25
2-Methyl-5-chloro-	..	85-90(1)	..	$C_9H_{12}ClN$	63·88	7·22	7·98	64·06	7·13	8·25
2-Chloro-5-methyl-	..	98-104(1·5)	..	$C_9H_{12}ClN$	64·32	7·08	8·16	64·06	7·13	8·25

TABLE 2

S. No.	R_1	R_2	R_3	R_4	R_5	R_6	M.P. °C.	Mol. formula	Analysis					
									Calc.			Found		
									C	H	N	C	H	N
1	H	H	H	H	CH_3	H	99	$C_{17}H_{16}N_2O$	77·25	6·1	10·60	77·28	6·36	10·48
2	Cl	H	H	H	CH_3	H	129-30	$C_{17}H_{15}N_2OCl$	68·32	5·06	9·38	68·44	5·32	9·4
3	H	Cl	H	H	CH_3	H	137-38	$C_{17}H_{15}N_2OCl$	68·32	5·06	9·38	68·26	4·98	9·21
4	H	H	Cl	H	CH_3	H	120	$C_{17}H_{15}N_2OCl$	68·32	5·06	9·38	68·18	5·16	9·08
5	OCH_3	H	H	H	CH_3	H	141	$C_{18}H_{18}N_2O_2$	73·45	6·16	9·52	73·60	6·28	9·3
6	H	H	OCH_3	H	CH_3	H	106	$C_{18}H_{18}N_2O_2$	73·45	6·16	9·52	73·16	6·08	9·3
7	H	H	CH_3	H	CH_3	H	108	$C_{18}H_{18}N_2O$	77·67	6·52	10·07	77·46	6·68	9·9
8	H	H	H	H	CH_3	Br	145	$C_{17}H_{15}N_2OBr$	59·50	4·41	8·15	59·42	4·36	8·3
9	OCH_3	OCH_3	H	H	CH_3	H	121	$C_{19}H_{20}N_2O_3$	70·35	6·22	8·64	70·4	6·41	8·5
10	H	OCH_3	OCH_3	H	CH_3	H	132-33	$C_{19}H_{20}N_2O_3$	70·35	6·22	8·64	70·28	6·4	8·6
11	H	OCH_3	OC_2H_5	H	CH_3	H	130	$C_{20}H_{22}N_2O_3$	70·98	6·5	8·2	7·0	6·4	7·9
12	Cl	Cl	H	H	CH_3	H	148	$C_{17}H_{14}N_2OCl_2$	61·28	4·04	8·41	61·06	4·2	8·3
13	H	Cl	Cl	H	CH_3	H	142	$C_{17}H_{14}N_2OCl_2$	61·28	4·04	8·41	61·3	4·1	8·35
14	CH_3	Cl	H	H	CH_3	H	102	$C_{18}H_{17}N_2ClO$	69·11	5·47	8·96	69·32	5·6	9·08
15	CH_3	H	H	Cl	CH_3	H	134	$C_{18}H_{17}N_2ClO$	69·11	5·47	8·96	69·06	5·38	8·9
16	Cl	H	H	CH_3	CH_3	H	104	$C_{18}H_{17}N_2ClO$	69·11	5·47	8·96	69·28	5·32	9·1
17	H	H	H	H	C_2H_5	H	95	$C_{18}H_{18}N_2O$	77·67	6·52	10·07	77·62	6·48	10·13
18	H	OCH_3	OCH_3	H	C_2H_5	H	86	$C_{20}H_{22}N_2O_3$	70·98	6·55	8·28	71·00	6·60	8·34

compounds of the present series, protected mice against maximum electric shock, and metrazole induced convulsions. Two of the compounds mentioned above were further studied.

Tranquillizing Effect

Behaviour of cats, monkeys, and adult mongrel dogs was observed before and after administration of test compounds. Animals were observed for spontaneous motor activity, gait, posture, behaviour towards observers, response to handling, interest in surroundings, aggressiveness, and autonomic functions like salivation, vomiting, diarrhoea, etc. Aggressiveness in cats was studied by observing its response to the presence of a mouse in the cage. Effects on behaviour patterns were observed continuously for a period of 6 hr and also after 24 hr.

R/G-5/2 at a dose of 10 mg./kg. i.p. injected into monkeys produced sedation, decrease in normal movement, and a tendency to lie down in the cage, and generalized loss of interest in the surrounding within half an hour. Effect lasted for 4 hr. In dogs, the same doses produced sedation and decreased movement. On provocation, the dog did get up, was quite awake, but settled down again immediately. There was no loss of muscle tone. A similar action was seen in cats at a dose of 20 mg./kg. given orally.

Compound R/G-5/5 showed similar activity but was found less potent. The animals recovered completely at the end of 24 hr.

Motor Activity in Mice

Spontaneous motor activity in mice was studied by recording the total activity of a group of 6 mice, in a photoelectric activity box (Actophotometer). Period of recording was 10 min. before, and 1, 2 and 4 hr after drug administration. The control group received only vehicle. The compounds were administered at a dose of 65 mg./kg. (1/10 LD_{50}). Methaqualone hydrochloride was tested at 40 mg./kg. (1/10 LD_{50}) (Table 3).

TABLE 3—SPONTANEOUS MOTOR ACTIVITY

(Six mice in a group; activity recorded for 10 min.)

No. of groups	Drug	Dose *mg./kg. i.p.*	Before	% Inhibition after the drug administration			Remarks
				1 hr	2 hr	4 hr	
3	Normal saline	0·1 ml./10 g.	835	24	36	40	Active
3	Methaqualone	40	631	77	86	54	Sedation ptosis
3	R/G-5/2	65	625	64	64	57	Sedation ptosis
3	R/G-5/5	65	557	53	65	42	Sedation ptosis

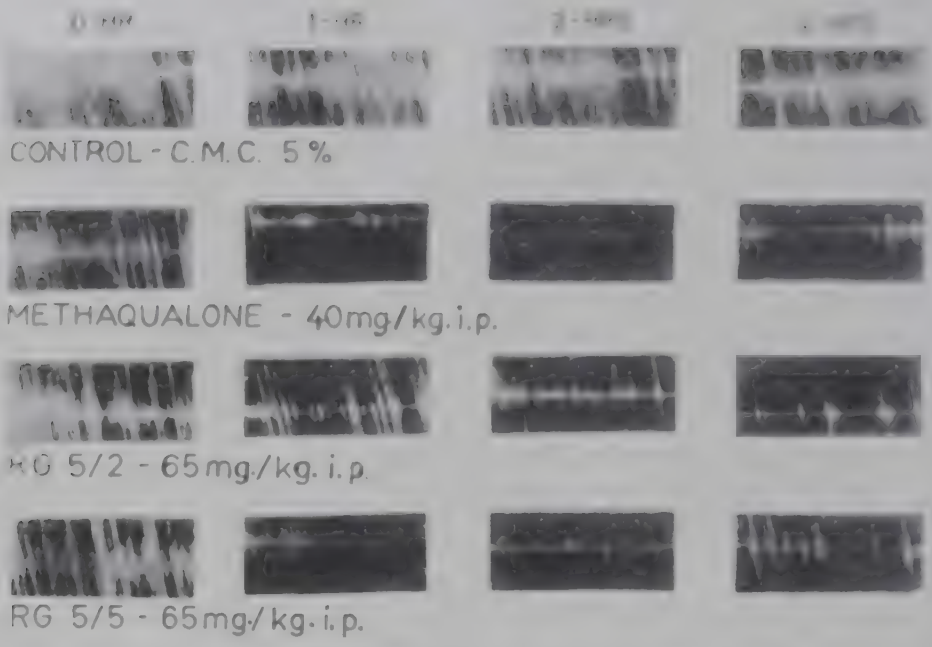


FIG. 2 — SPONTANEOUS MOTOR ACTIVITY (ACTOGRAPH)

The movements were markedly diminished. The peak effect was observed after 2 hr. R G-5 2 produced greater inhibition than R G-5 5. There was a greater loss of muscle tone with methaqualone hydrochloride than R G-5 2 and R G-5 5. There was marked blepharospasm. Spontaneous activity in mice was also measured in spring mounted cages (Actograph). Movements were recorded graphically on smoked paper. A mouse was injected i.p. with solvent, and placed in the recording cage. The activity for the following 10 min. was recorded. After this control observation the mouse was injected with the test compounds (65 mg./kg. i.p.). Methaqualone hydrochloride was used for comparison at a dose of 40 mg./kg. i.p. Movements were recorded 1, 2 and 4 hr after injection. The control group received only solvent (CMC 0·5%). Three mice per compound were studied. There was marked decrease in activity with all the compounds (Fig. 2). Mice injected with R G-5 2 and R G-5 5 showed sedation without any ataxia and tended to recover after 4 hr whereas those with methaqualone hydrochloride showed sedation, were markedly ataxic, and the effects persisted well beyond 4 hr.

Orientation Hyperactivity

This was studied in adult mice, according to the method described by Cutting *et al.*[11]. The total activity for 30 min. was recorded. The recorded activity was compared to saline treated controls and expressed as percentage inhibition (Table 4).

Compound 5 2 inhibited the activity by 61 per cent; compound 5 5 by 49 per cent and meprobamate at a dose of 100 mg./kg. showed 39 per cent inhibition.

TABLE 4—ORIENTATION HYPERACTIVITY IN MICE

(Six mice per group)

No. of groups	Drug	Dose *mg./kg. i.p.*	Total activity for 30 min. ± S.E.	% Inhibition
3	Normal saline	0·1 ml./10 g.	1797±167	..
3	Methaqualone	40	147±9	92
3	R/G-5/2	65	704±69	61
3	R/G-5/5	65	925±53	49

TABLE 5—AMPHETAMINE INDUCED HYPERACTIVITY IN MICE

(Six mice per group)

No. of groups	Drug & Dose *mg./kg. i.p.*	Amphetamine sulphate 4 *mg./kg. s.c.*	Cumulative motor activity for 10 min. (expressed as per cent of control)	% Inhibition
3	Normal saline 0·1 ml./10 g.	..	100	..
3	Normal saline 0·1 ml./10 g.	Injected	195	..
3	Methaqualone, 40 mg.	Injected	96	99
3	R/G-5/2 65 mg.	Injected	145	50
3	R/G-5/5 65 mg.	Injected	163	32
3	Meprobamate, 100 mg.	Injected	163	32

Effect on Amphetamine Induced Hyperactivity

The stimulating effect of amphetamine (4 mg./kg. s. c.) on spontaneous motor activity in mice, and the effect of test compounds injected i.p., at a dose of 65 mg./kg., 90 min. prior to amphetamine, was recorded by Actophotometer. Total activity of each group (6 mice per group) was recorded for 10 min. (Table 5.)

Amphetamine increased activity to 195, when control was taken as 100. Compound 5/2 reduced this activity by 50 per cent; compound 5/5 decreased by 32 per cent and meprobamate at 100 mg./kg. reduced the activity by 32 per cent.

Oxygen Consumption

Oxygen consumption test was carried out, as suggested by Cattell *et al*.[14]. Time required for consumption of oxygen from 4 ml. of air per mouse, was recorded. Ten mice were tested per each compound, at a dose of 80 mg./kg. before, and 60 min. after administration of the compound (Table 6).

There was a decrease in oxygen consumption with both compounds R/G-5/2 and R/G-5/5, which was comparable with meprobamate at a dose of 100 mg./kg.

Hexobarbitone Sleep

Male rats weighing between 110 and 130 g. were injected with test compounds at a dose of 80 mg./kg. i.p. and followed 60 min. later by 100 mg./kg. of sodium hexobarbitone. The duration of loss of righting reflex was recorded (Table 7).

Compound 5/2 and 5/5 potentiated the anaesthetic activity of hexobarbitone at a significant level. The results are comparable with meprobamate.

TABLE 6—COMPARATIVE OXYGEN CONSUMPTION IN MICE

Drug & Dose *mg./kg. i.p.*	Seconds to consume 4 ml. of air average of 10 measures		Difference	Symptoms
	Before	After		
Control	180	190	+10	Active, moving all over
Meprobamate, 100 mg.	175	211	+36	Mild sedation
R/G-5/2, 80 mg.	170	195	+25	Mild sedation & ptosis, decrease in activity
R/G-5/5, 80 mg.	175	210	+35	Mild sedation

TABLE 7—HEXOBARBITONE SLEEPING TIME

(Hexobarbitone sodium solution 10 mg./ml.)

Drug & Dose *mg./kg. i.p.*	Hexobarbitone *mg./kg. i.p.* (after the drug 90 min.)	No. of rats	Sleeping time *min., mean ± S.E.*	'P' Value
Control	100	6	24 ± 0·84	..
Meprobamate 100 mg.	100	6	36 ± 1·60	0·01
R/G-5/2, 80 mg.	100	6	37 ± 1·90	0·01
R/G-5/5, 80 mg.	100	6	31 ± 2·70	0·05

Amphetamine Toxicity in Mice

Adult albino mice in groups of 10 were kept in cubical wire mesh cages (16 cm.), after receiving subcutaneously, 14 mg./kg. of *d*-amphetamine sulphate. Other groups received in addition a test compound (65 mg./kg. i.p.) of physiological saline 90 min. prior to amphetamine. Deaths were counted in 24 hr.

d-Amphetamine sulphate produced 100 per cent mortality in aggregated mice within 24 hr. There was no significant protection by any of the test compounds. Meprobamate at 100 mg./kg. also failed to protect mice.

Analgesic Effect

Analgesic effect was investigated by a number of tests like 'rats tail flick method', 'hot plate method', 'clip method' and 'Nilsen's method,' using an electrical stimulus, applied to a mouse tail producing a squeak response. No analgesic effect could be demonstrated with R/G-5/2 and R/G-5/5. These compounds also failed to potentiate analgesic effect of pethidine hydrochloride.

Anticonvulsant Activity

Anticonvulsant activity of test compounds was studied using maximum electric shock test (MES) and Metrazol seizure threshold test (MET) described by Swinyard, Brown, and Godman[15]. Ten mice were used for each test and tests were carried out 1, 2 and 4 hr after administration of test compound (65 mg./kg. i.p.). Methaqualone hydrochloride was also tested at a dose of 40 mg./kg. i.p. Compound R/G-5/2 and R/G-5/5 failed to protect mice against MES and chemically induced seizures. Methaqualone hydrochloride showed potent anticonvulsant activity by both these tests.

Anti-inflammatory Activity

Canageenan induced oedema was produced in rat's paw. Compound 5/2 showed mild anti-inflammatory activity as measured by inhibition of oedema, compared to hydrocortisone and phenylbutazone.

Antitussive Activity

Superior laryngeal nerve in cats was exposed under pentobarbitone sodium anaesthesia (30 mg./kg. i.p.). The nerve was electrically stimulated (2 volts, pulse with 10×10 m.sec., frequency 5/sec.) and cough response was recorded on a kymograph (Fig. 3). Codeine phosphate in a dose of 2 mg./kg. i.v. almost immediately blocked the cough response. The effect was well maintained till 60 min. Compound 5/5 in a dose of 5 mg./kg. inhibited the cough response partially for 30 min. Compound 5/2 in a dose of 2 mg./kg. i.v. inhibited the cough response and inhibition lasted for more than 90 min.

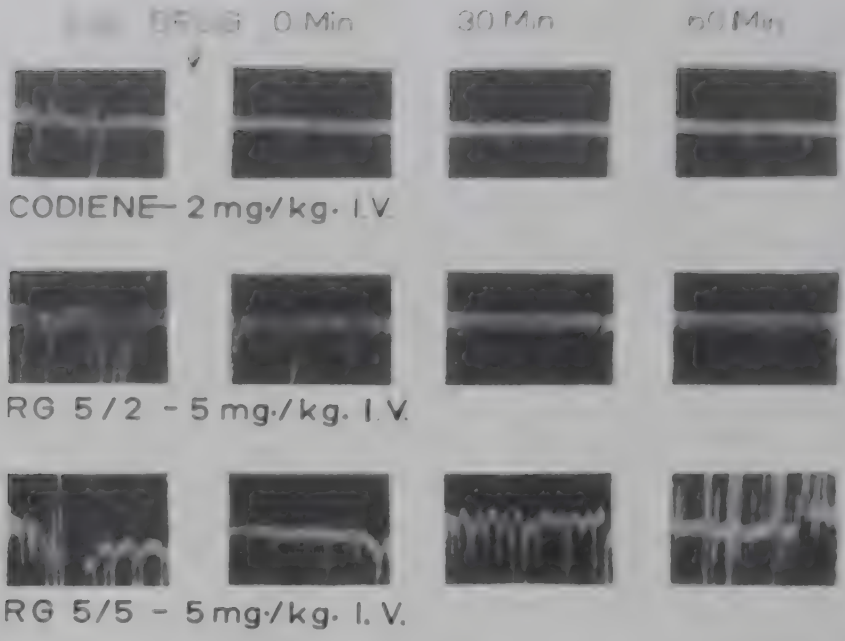

0 Min
30 Min
CODIENE— 2 mg./kg. I.V.
RG 5/2 - 5 mg./kg. I.V.
RG 5/5 - 5 mg./kg. I.V.
E.S. – ELECTRICAL STIMULATION OF SUPERIOR LARYNGEAL NERVE; VOLTAGE - 2, WIDTH - 10 x 10 m.sec., FREQ - 5 c.p.s

FIG. 3 — CAT-ANTITUSSIVE EFFECT

Effect on guinea-pig trachea. A guinea-pig tracheal chain preparation was set up (Fig. 4). Effect of the test compound was tested against acetylcholine and histamine induced spasm. Both the compounds decreased response to acetylcholine and histamine. Compound 5·2 in concentration of 0·02 μg. ml. inhibited acetylcholine response more than that to histamine.

Conditioned Avoidance Response CAR

CAR was established in rats using Cook's pole climbing technique (Cook and Weidley[16]. Under the effect of the test compounds though there was no inhibition of CAR, the latency response to a conditioned response was appreciably prolonged.

Effect on reserpine treated mice. Test compounds were injected i.p. (65 mg. kg.) in adult albino mice and 2 hr later reserpine 2·5 mg. kg. i.p. was injected. The action of reserpine was potentiated.

Rectal Temperature

Effect of test compounds on the rectal temperature of adult male rats, recorded with a thermometer was studied for 4 hr (Fig. 5). Compound 5·2 produced a significant fall in the rectal temperature at the end of 2 hr as compared to control.

Local anaesthetic activity. Local anaesthetic activity was studied on rabbit's cornea with the test compounds in varying concentration of 0·5 to 10·0 per cent. No local anaesthetic property could be demonstrated.

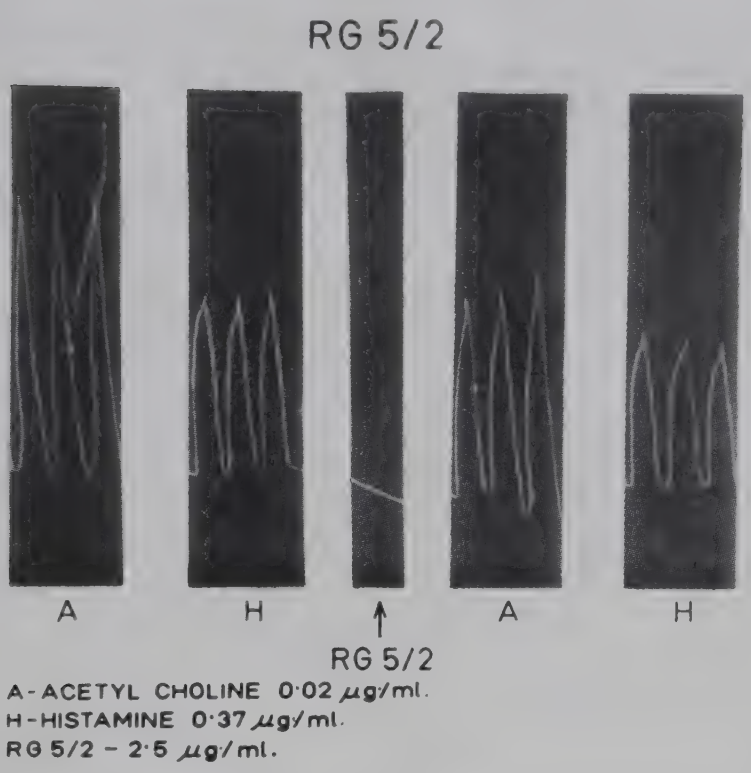


FIG. 4 — GUINEA-PIG TRACHEAL CHAIN

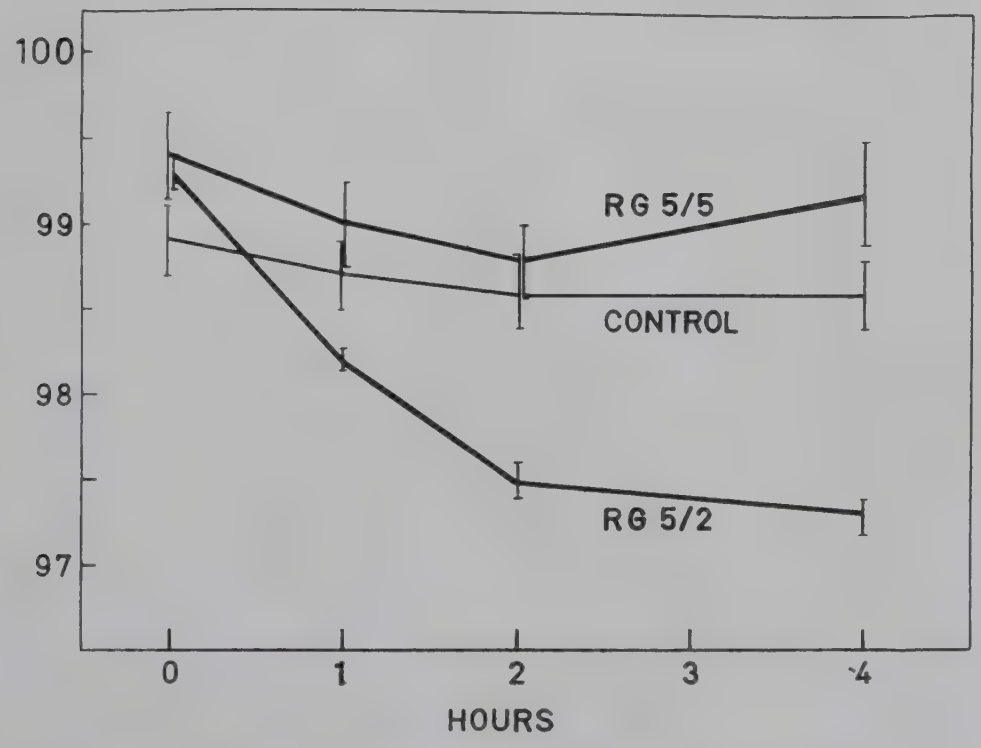


FIG. 5 — MEAN OF RECTAL TEMPERATURES OF 10 RATS AND S.E.

Acute toxicity. Acute toxicity was studied in white mice. The test compounds were injected i.p. and LD_{50} in terms of mg./kg. was calculated (Litchfield and Wilcoxon[17]). LD_{50} of methaqualone was found to be 530 mg./kg. (200 to 400), 5.2 was 650 mg./kg. (400 to 800) and 5.5 was 580 mg./kg. (400 to 800).

Effect on frog rectus muscle. Effect of the test compound on acetylcholine induced contraction of frog's rectus abdominis was studied. Compound 5.2 produced about 10 per cent reduction in the acetylcholine response, which was reversible.

Effect on smooth muscle. Compound 5.2 and 5.5 reduced acetylcholine response on guinea-pig ileum in concentration of 10 μg./ml. Action of 5HT on rat colon was also reduced by both these compounds in the same concentration. Both these effects were reversible.

Action on isolated rabbit heart (Langendroff preparation). Effect on test compounds on the force and rate of contraction of rabbit's isolated heart was studied in a Langendroff preparation. At a concentration of 20 μg./ml. and above, there was a decrease in force of contraction and the heart rate.

Action on blood pressure and respiration. Carotid blood pressure was recorded in dogs anaesthetized with pentobarbital sodium (35 mg./kg. i.p.). Test compounds were suspended in 0·5 per cent of CMC and injection i.v. in a dose of 10 mg./kg. Effect on blood pressure, carotid occlusion reflex and response to injections of adrenaline (1 μg./kg.), noradrenaline (1 μg./kg.), acetylcholine (1 μg./kg.), and histamine (1 μg./kg.) was noted. These observations were repeated at 30 min. interval for 4 hr after injection of test compounds. Carotid occlusion response was diminished. There was no change in response to adrenaline, noradrenaline, acetylcholine and histamine.

Antistress effect. Effect on adrenal ascorbic acid and cholesterol in rats under standardized centrifugal stress applied in a vertical plane was noted (Pohujani[18]. This stress depleted both adrenal ascorbic acid and cholesterol. The effect of test compounds, meprobamate and phenobarbitone in blocking this effect is shown in Table 8. Phenobarbitone and compound 5.2 significantly blocked the depletion of adrenal ascorbic acid and cholesterol. Compound 5.5 blocked only depletion of ascorbic acid.

DISCUSSION

The present series of quinazolines are significant from the point of introduction of a sympathomimetic amine into a quinazoline moiety. Amine, Mehta & Samarth (First Int. Pharm. Conference, page 146, abstract 488, [1962]) claimed powerful bronchodilator activity for the quinazoline moiety. It is also well known that many of the sympathomimetic amines are bronchodilators. An attempt was made in the present investigation to

TABLE 8 EFFECT ON RAT ADRENAL ASCORBIC ACID AND CHOLESTEROL AFTER STRESS

Drug & Dose *mg./kg.*	No. of animals	Adrenal ascorbic acid (mg. per 100 g. of tissue with S.E.)	Adrenal cholesterol (mg. per g. of tissue with S.E.)
Normal	13	445·0± 4·5	33·65±1·5
Control	16	252·0± 9·1	22·13±0·61
Meprobamate, 100	6	290·6±21·8	23·07±0·58
Phenobarbitone, 5·0	5	* 351·3±11·0 (P. 0·01)	* 25·96±0·26 (P. 0·01)
R/G-5/2, 80	6	* 315·8±11·8 (P. 0·01)	* 23·59±0·27 (P. 0·05)
R/G-5/5	4	* 290·3±11·7 (P. 0·02)	23·29±0·50

*Significant

find out the changes produced in the activity in quinazolines due to fusion of the sympathomimetic moiety.

Quinazolines are reported to have a number of pharmacological effects, such as hypnotic, analgesic, antitussive, anticonvulsant, anticholinergic, muscle relaxant and anti-inflammatory property. Clinically methaqualone [2-methyl-3-ortho-tolyl-4 (3*H*) quinazolinone] has been introduced as nonbarbiturate hypnotic. Swift *et al.*[19] reported potent anticonvulsant and central muscle relaxant property with the same compound.

The present series of quinazolines differ in many respects from the compounds reported so far. Compared to methaqualone LD_{50} of compounds 5/2 and 5/5 was found to be nearly 100 per cent higher. The hypnotic property of the compounds 5/2 and 5/5 was much less compared to methaqualone. At comparable doses of 1/10, LD_{50} of compounds 5/2 and 5/5 produced more sedation and tranquillization without loss of muscle tone as compared to methaqualone. The decreased spontaneous activity observed in mice due to the methaqualone was associated with marked ataxia. This series of quinazolines did not show any anticonvulsant activity which is in contrast to potent anticonvulsant activity seen with most of the other quinazoline derivatives. Methaqualone hydrochloride has been reported to possess local anaesthetic activity both on rabbit cornea and intradermal route. Compounds R/G-5/2 and 5/5 failed to show any local anaesthetic activity. Methaqualone hydrochloride produces transient fall in B.P. associated with myocardial depression. Compounds 5/2 and 5/5 did not produce any fall in blood pressure. However, they did produce a decrease in carotid occlusion response which may be due to central depressant action. At higher dose they produced myocardial depression. In cats, compound 5/2 showed potent antitussive activity against electrical stimulation of superior

laryngeal nerve. This activity was comparable to that of codeine. Compound 5/5 produced lesser degree of inhibition, lasting for the shorter period. Compound 5/2 also showed interesting antichodinergic and antihistaminic activity on guinea-pig tracheal chain. Against carrageenan induced oedema in rat paw, compound 5/2 showed a mild anti-inflammatory activity.

Becker and Hays[20] and Cass and Frederik reported potentiation of codeine analgesia with methaqualone. Compounds 5/2 and 5/5 failed to potentiate pethidine analgesia in animals.

Compound 5/2 because of its sedative, tranquillizing, antitussive and mild anti-inflammatory properties—at a dose which does not produce sleep and ataxia, seems promising for further studies.

Pilot clinical trials have been planned and will be started as soon as the results of subacute toxicity are made available. This compound will be investigated both as a tranquillizer and as an antitussive.

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The authors sincerely thank Mrs K. Bhanumathi for technical assistance during the course of the work.

REFERENCES

1. Laubach G. D. & McLamore, W. M., U.S. Pat. 2, 915, 521 (1959); C.A., **55** (1961), 25998.
2. Boissier, J. R. & Picard, Y. F., *Therapie*, **15** (1960), 57; C.A., **57** (1962), 6564.
3. Boissier, J. R. & Pagny, J., *Med. Exptal.*, **1** (1959), 368; C.A., **54** (1960), 21460.
4. Wallace & Tiernan Inc., Br. Pat. 914, 630 (1963).
5. Bhaduri, A. P., Khanna, N. M. & Dhar, M. L., *J. sci. indust. Res.*, **21 B** (1962), 378.
6. Wallace & Tiernan Inc., Br. Pat., 912, 085 (1962); C.A., **58** (1963), 10217.
7. Scarborough, H. C., U.S. Pat. 3, 073, 826 (1963); C.A., **59** (1963), 1656.
8. Kacker, I. K. & Zaheer, S. H., *J. Indian chem. Soc.*, **28** (1951), 344.
9. Duchastel, Y., *Union Med. Canada*, **91** (1962), 288; C.A. **56** (1962), 14894.
10. Bianchi, C. & David, A., *J. Pharm. Pharmac.*, **12** (1960), 501.
11. Societe Industrielle Pour la Fabrication des Antibiotiques (S.I.F.A.), Belg. Pat. 611, 980 (1962); C.A., **59** (1963), 1655.
12. Beech, W. F., *Soc.*, (1954) 1297.
13. Grimmel, H. W., Guenther, A. & Morgan, J. F., *J. Am. chem. Soc.*, **68** (1946), 543.
14. Cutting, W., Parkman, H., Read, G., Read, D., Cutting, J. & Furst, A., *Int. Pharmacodyn.*, **121** (1959), 14.
15. Swinyard, E. A., Brown, C. W. & Goodman, L. S., *J. Pharmac. exp. Ther.*, **106** (1952), 319.
16. Cook, L. & Weidley, E. F., *Ann. N. Y. Acad. Sci.*, **96** (1957), 315.
17. Litchfield, J. T. (Jr) & Wilcoxon, F., *J. Pharmac. exp. Ther.*, **96** (1949), 99.
18. Pohujani, S. M., Thesis for M.Sc. degree, University of Bombay, 1965.
19. Swift, J. G., Dickens, E. A. & Becker, B. A., *Arch. int. Pharmacodyn.*, **128** (1960), 153.
20. Becker, B. A. & Hays, E. E., *J. Pharmac. exp. Ther.*, **99** (1958), 17.

Pharmacological Aspects of MAOI Antidepressors : Quantitative Interactions with Reserpine and DOPA

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At first sight, the pharmacological methods for studying MAOI seem numerous (Boissier, 1965; Simon, 1965); in fact only three large groups of methods are at present capable of yielding satisfactory results. These methods depend on obtaining evidence either of the antireserpine activity, or of the anticataleptic activity, or of properties concerning the enzymatic action, diminution of tissular MAO activity, modification of tissular level in bio-amines, potentiation of tryptamine action and potentiation of the action of bio-amine precursors (DOPA for catecholamines, 5HTP for serotonin).

In each of these groups of methods, the variations and imprecisions in the procedures used make it difficult to compare results obtained by different authors.

During a systematic study concerning a few MAOI belonging to different chemical series, we have had to go thoroughly into certain technical points and we would like to mention here three particular problems we came across:

(1) the most favourable conditions for obtaining evidence of MAOI antagonistic activity towards reserpine hypothermia

(2) the quantification of the inversion by MAOI of the activity of high doses of reserpine on the behaviour of mice

(3) the particular phenomena met with during the study of MAOI-DOPA interaction, phenomena which allow an explanation for a certain number of seemingly paradoxical results found in literature

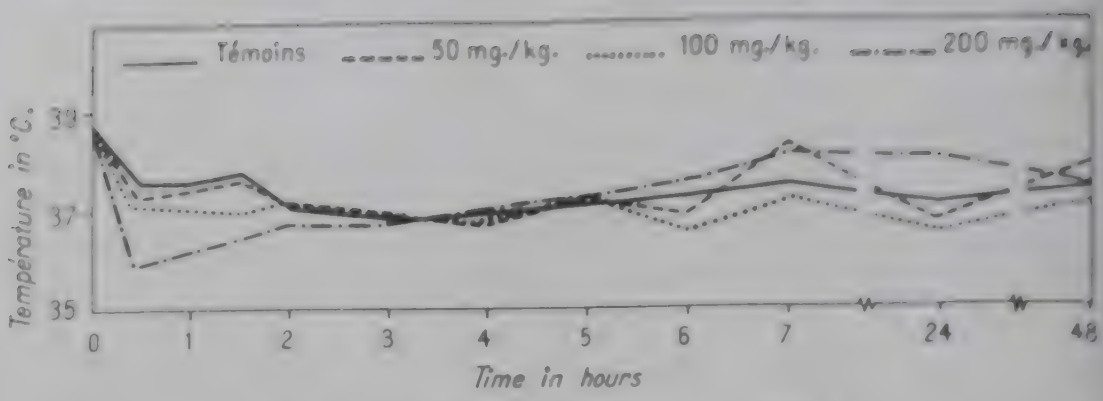

FIG. 1 — PARGYLINE GIVEN ORALLY AT TIME O

MAOI and Reserpine Hypothermia

However, if almost all the pharmacological actions of reserpine can be successfully antagonized by MAOI, a preference usually goes to the study of ptosis and hypothermia.

Hypothermia due to medium doses of reserpine develops slowly and reaches an optimum level after 24 hr, before increasing again. Two factors are particularly important to study the antagonism of this action by MAOI: the time of administering the MAOI compared with that of administering reserpine, and the dose of reserpine.

We have chosen pargyline as an example as it does not modify the temperature of mice (Fig. 1).

Methods

All experiments were performed on male mice, weighing 18 to 22 g, of homogeneous breed, in a climatized laboratory (22 ± 1°C.). Temperature measurements were performed with a rectal catheter, pushed to constant depth, carrying a thermoelectric couple, connected to a reading apparatus graduated in 0, 1°C.

Different procedures were used (for details, see key to figures).

A. MAOI Administered before Reserpine

Single administration of MAOI. Pargyline is given orally 24 hr before administration of reserpine i.p. The study of Fig. 2, which represents the results obtained compared to the dose of reserpine shows that the most satisfactory results are obtained with 3 mg./kg. of reserpine; with inferior doses the action is not clear, or is not proportional to the dose of MAOI; with superior doses, reserpine hypothermia, although not much clearer, is more difficult to antagonize.

Repeated administration of MAOI. Repeated administration of pargyline during eight days also gives satisfactory results (Fig. 3). These conditions are certainly nearer to conditions of therapeutic use of MAOI (repeated administration of weak doses). From a purely technical point of

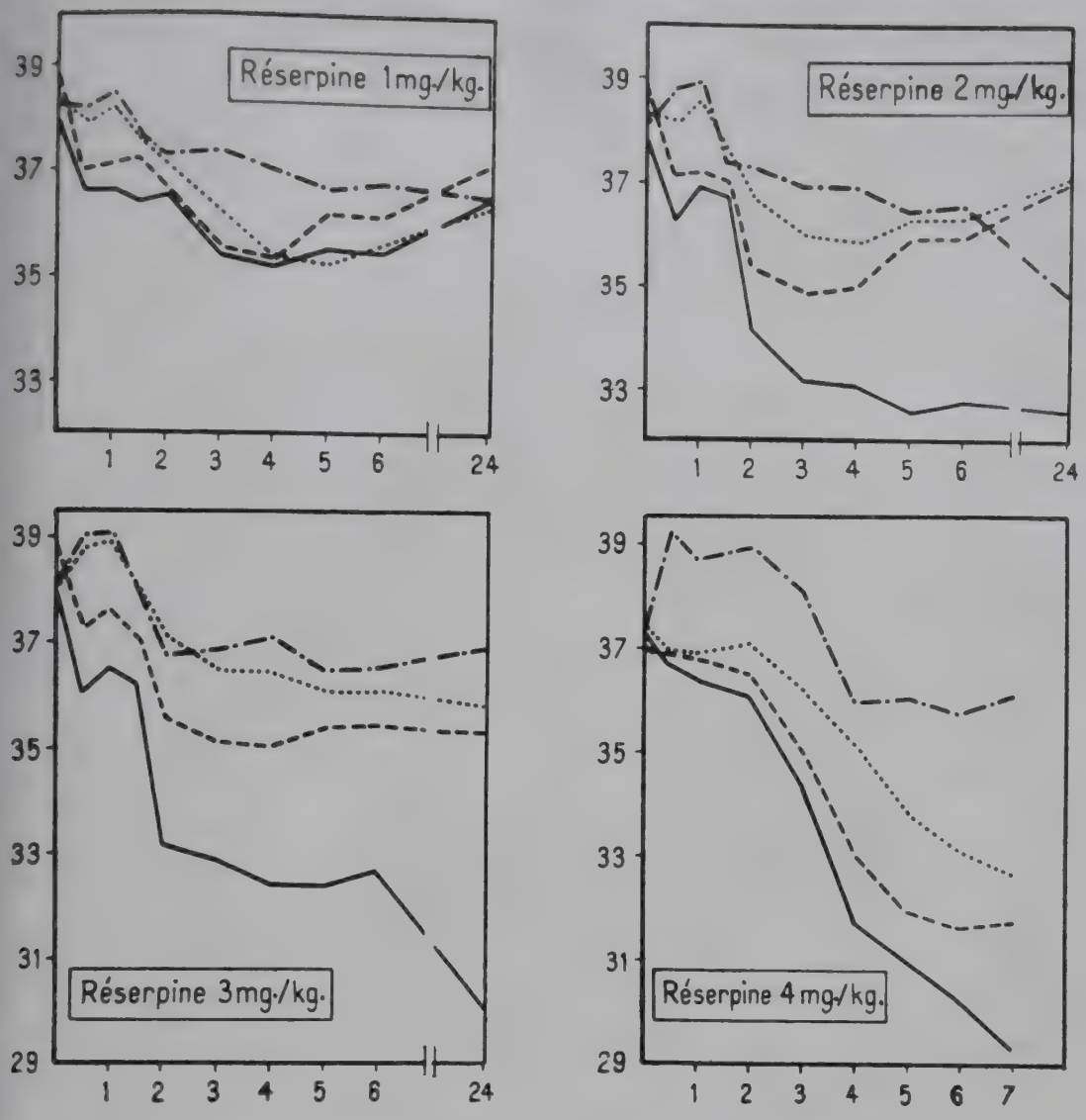

FIG. 2—PARGYLINE GIVEN ORALLY 24 HR BEFORE I.P. ADMINISTRATION OF RESERPINE [Doses indicated in front of each group of curves: Doses of pargyline, ——0 mg./kg.; - - - 50 mg./kg.; . . . . 100 mg./kg.; -·-·-200 mg./kg.]

view, the necessity of treating regularly the animals represents a complication that more interesting results with this test do not counterbalance.

B. **MAOI Administered at the Same Time as Reserpine**

The antagonism due to MAOI is clear, especially from the third hour onwards (Fig. 4.)

C. **MAOI Administered after Reserpine**

The results obtained are not so clear, except at a dose of 200 mg./kg. of pargyline (Fig. 5). Further, the increase in temperature of control animals

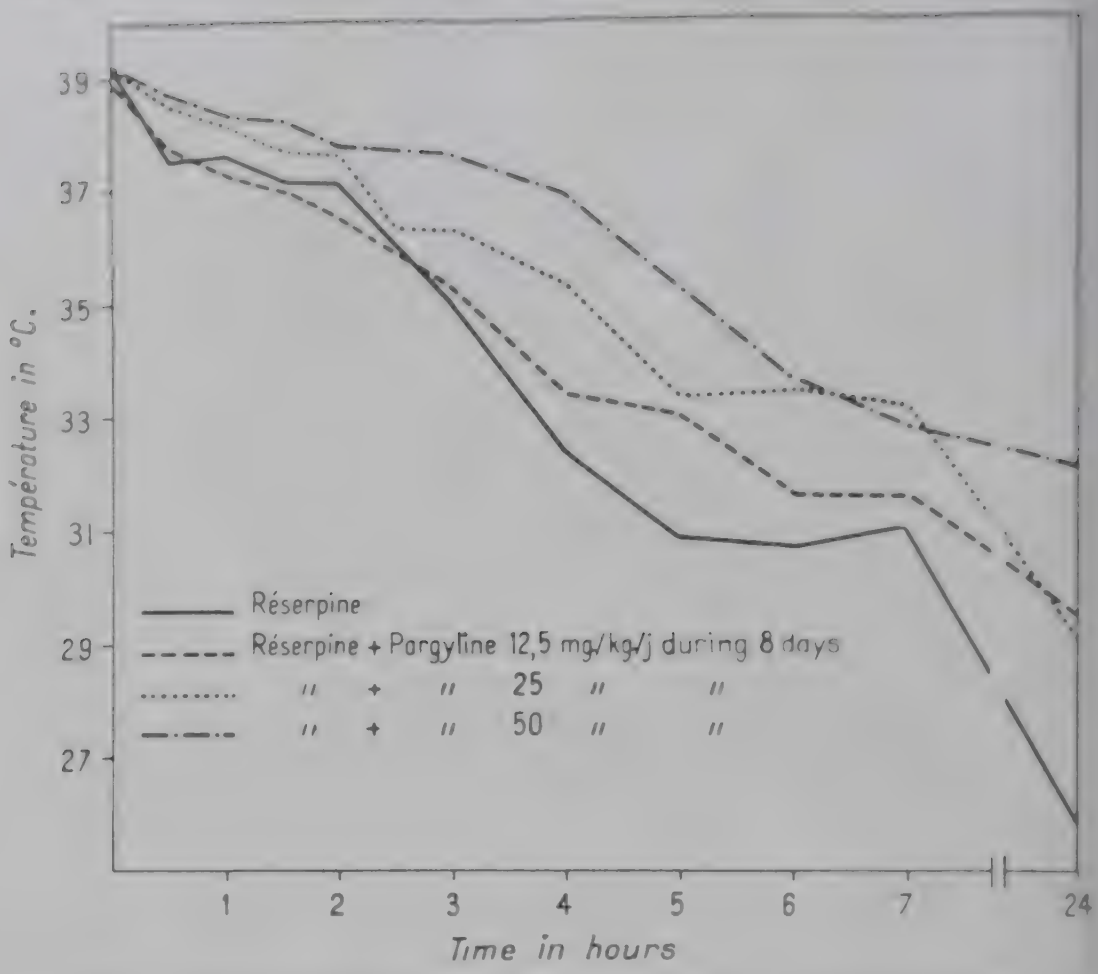

FIG. 3 — PARGYLINE GIVEN ORALLY EVERY DAY FOR 8 DAYS [On the 9th day, animals received (time 0) reserpine 3 mg./kg. i.p.]

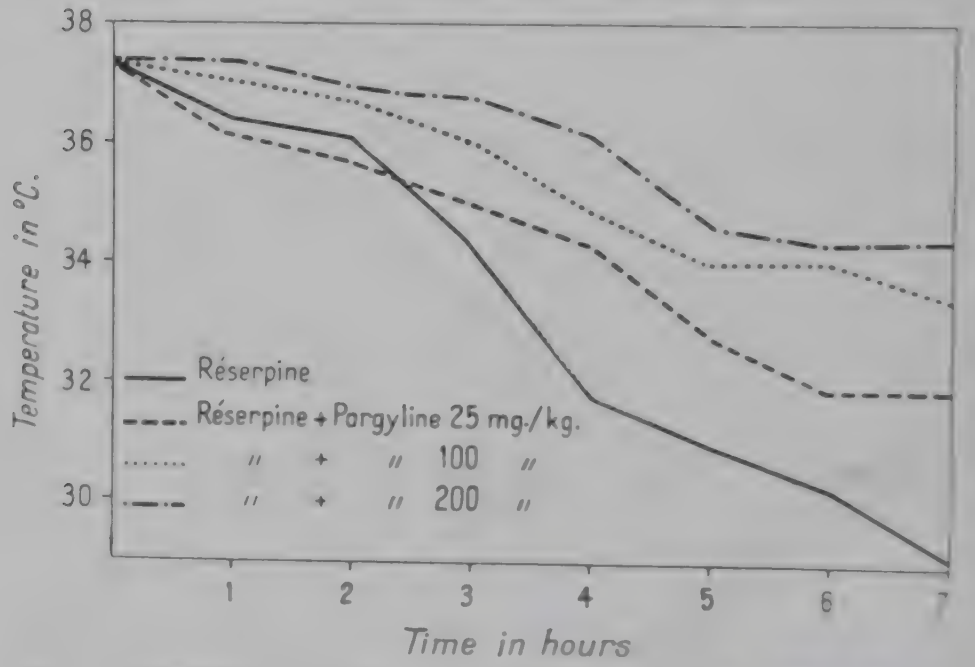

FIG. 4 — RESERPINE (3 mg./kg. i.p.) ADMINISTERED AT THE SAME TIME AS PARGYLINE ORALLY

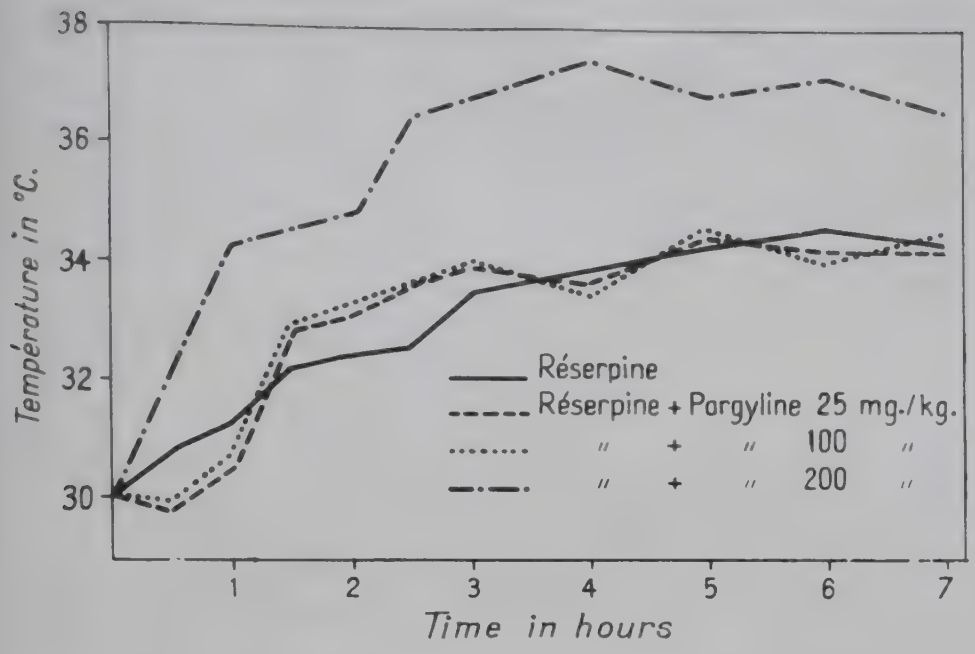

FIG. 5 — PARGYLINE ADMINISTERED ORALLY 18 HR AFTER RESERPINE (3 mg./kg. i.p.)

is probably more due to frequent manipulation necessary with these very hypothermic animals, than to the disappearing of reserpine action.

The major drawback of B and C methods is that certain MAOI possess, in addition to their enzymatic inhibitory action, a quickly appearing amphetamine-like action; and amphetamine partly opposes the hypothermia induced by reserpine. It is therefore not possible with these techniques to determine whether the activity put into evidence is due to the direct action of MAOI, or to one of their side effects sometimes encountered.

From the practical point of view, we believe that the most favourable conditions for studying the phenomena are as follows: single dose of MAOI, given orally; 24 hr later, i.p. administration of reserpine (3 mg./kg.).

Reversal of Action of Reserpine on the Behaviour of Mice by MAOI

Many authors have pointed out that previous administration of MAOI could result in a truly reverse action of reserpine; instead of the usual picture, sometimes very important motor excitation is noticed, associated with mydriasis, horripilation, hyperthermia, tachycardia (Besendorf and Pletscher, 1956; Brodie *et al.*, 1956; Chessin *et al.*, 1957; Everett *et al.*, 1959; Gylys *et al.*, 1963; Smith and Dews, 1962). The most generally set forth explanation for this 'reversal activity' is biochemical; the administration of a high dose of reserpine leads to a sudden liberation of catecholamines which have, since they are not metabolized because of the inhibition of the MAO, an exciting action.

The phenomena observed are very clear, and their simple observation

has been suggested as a method to compare the power of different MAOI. If such a qualitative method can give good results when performed by one same experimentator, the published results can hardly be compared and used from one laboratory to another.

That is why we have tried to quantify the phenomena thanks to an actographical method.

Method

We use naive male mice, weighing 18 to 22 g. The actograph used of Dews type (Dews, 1953) has been previously described (Boissier and Simon, 1965a); the movements of the animals are quantified by photoelectric cells connected to recorders.

Preliminary experiments have shown us the necessity of administering the MAOI studied for a few days *before the test* and using large doses of reserpine (8 mg./kg. i.v. or 16 mg./kg. i.p.). The results shown in Fig. 6 were obtained as follows: Oral administration daily for eight days of the substance to be studied, followed by i.p. injection of reserpine (16 mg./kg.) and measurement of the motor activity of the mice from the 30th to the 40th min. after reserpine.

The results obtained by this method with different MAOI are therefore very satisfactory and permit a comparative assessment of these substances. Furthermore, the specificity of this method is particularly interesting because with these experimental conditions, imipramine-like drugs and amphetamine have no action.

MAOI-DOPA Interactions

The stimulating action of dihydroxyphenylalanine (DOPA) on animals pretreated with MAOI is often used for the pharmacological study of these antidepressors. Evidence of this stimulating action is direct (motor excitation, hyperthermia) or indirect (antireserpine or antisleep activities). If mention is often made of 'potentiation' of DOPA activity by MAOI, the timeliness of using such a term does not seem evident after an attentive review of literature; many in fact are the contradictions concerning the activity of DOPA in normal animals.

DOPA has, according to different authors, either a stimulating or a depressing activity, and for some it has no action on behaviour, even with large doses (Boissier and Simon, 1965).

The multiplicity of experimental conditions (species, sex and age of animals, administration route, laevo-rotatory or racemic DOPA, time of observation, chosen criterion) can certainly partly explain these contradictions, but nevertheless certain obscurities do remain.

To try and sort out the problem we have undertaken, under well precised experimental conditions, a systematic study of MAOI-DOPA association in mice.

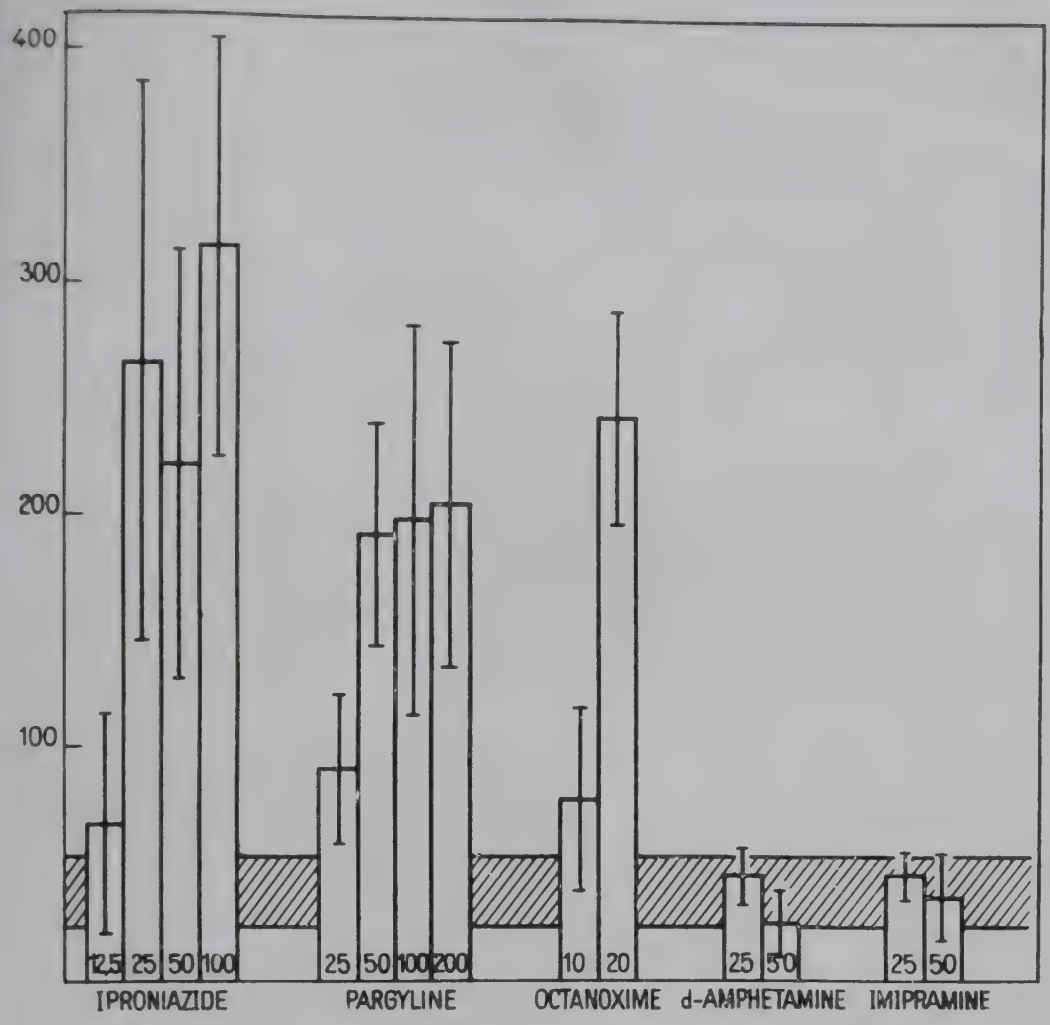

FIG. 6 — MOTOR ACTIVITY OF MICE (12 PER GROUP) RECEIVING ORALLY EVERY DAY FOR 8 DAYS THE INDICATED DOSE (mg./kg.) OF THE DRUG [On the 9th day, all the animals receive reserpine (16 mg./kg. i.p.) 30 min. before the test. Every mouse is put in the actograph. Height of the ranges 4 standard deviation to the mean (+2 SM.) The zone in grey corresponds to animals receiving only reserpine. Controls, receiving only isotonical solute of NaCl crossed 111, 4±8, 9 beams during 10 min.]

MATERIALS AND METHODS

Male mice of homogeneous breed, weighing 18 to 22 g. were employed.

dl 3, 4-Dihydroxyphenylalanine (Fluka) was always administered i.p. in aqueous solution (0·5 ml./20 g. body weight), except for doses above 500 mg./kg. without ever going beyond 1 ml./20 g.)

The MAOI used (iproniazid, pargyline, octamoxine) were always administered orally (0·5 ml./20 g. of weight) 20 to 25 hr before the test.

Actographical study. Mice motility was registered by the mean of the actograph previously described. During these experiments we have always put one single mouse per box for a time-length of 10 min. (with a minimum of 12 mice per dose). A preliminary study of DOPA activity

cucles showed that the optimal time for this measure was in between 13 and 25 min. following administration.

Potentiation of pentobarbital activity. All animals (8 mice per dose) receive i.p. an infrahypnotic dose of pentobarbital (27.5 mg./kg.). DOPA is administered i.p. 20 min. before the pentobarbital. The sleep criterion used was loss of righting reflex.

RESULTS

Actographical Study

The results obtained with a large variety of doses of DOPA are shown in Fig. 7. Animal behaviour changes can be sketched out as follows:

(a) Doses under 150 mg./kg. — no noticeable change.

(b) Doses in between 150 and 400 mg./kg. — in most cases the animals stay still (usually in vertical position against the corners of the boxes), they seem constantly awake, eyes open, aggressive towards the experimentator, hard to catch, also noticed are horripilation and tachypnea.

(c) Doses above 400 mg./kg. — animals are extremely agressive towards their neighbours, the experimentator, and any exterior object. Their movements are non-stop, jerky, very fast, coordinated and attentive, but do not seem to be motivated; however, the animals stay constantly 'all there', their hypermotility does not evoke at all an automatic phenomenon.

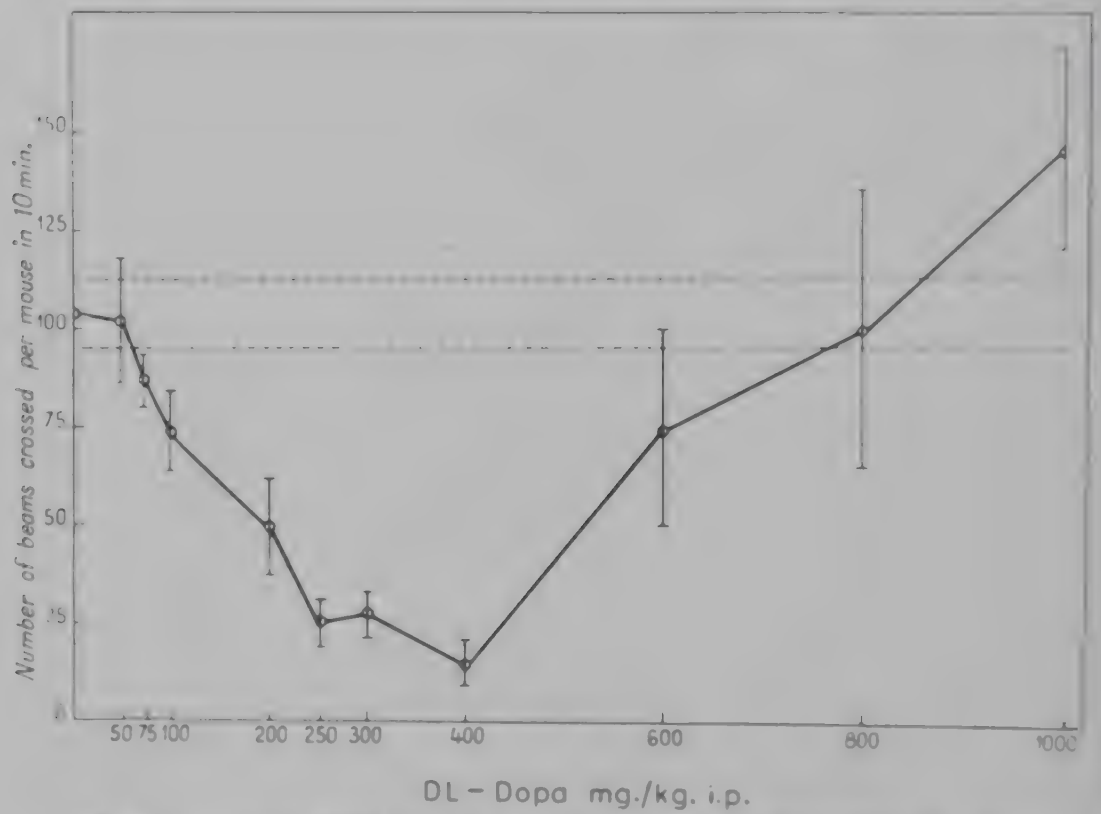

FIG. 7 — ACTION OF DOPA ON MICE MOTILITY [DOPA is administered 15 min. before beginning the test]

Considering this seemingly diphasic activity of DOPA, we have chosen to study the influence of MAOI pretreatment on the activity of the highest dose of DOPA still inactive on motility (75 mg./kg.) and of the highest 'depressing' dose still not producing hypermotility (400 mg./kg.).

The results, shown in Fig. 8 and which, considered by themselves, may seem at first paradoxical, become very easy to understand if one considers the behaviour of the animals.

(d) Mice, pretreated with large doses of MAOI and receiving 75 mg./kg. of DOPA react like normal animals treated with a dose of 300 to 400 mg./kg. of DOPA.

(e) Mice, pretreated with large doses of MAOI and receiving 400 mg./kg. of DOPA, react like normal animals treated with very large doses of DOPA; however, movements seem more 'automatic' and aggressiveness clearly fades. Furthermore, high lethality is noticed in the following hours.

For confirmation, we have studied the action of a very large variety of doses of DOPA on animals treated with a fixed and large MAOI dose (pargyline 150 mg./kg. orally). The diphasic curve obtained (Fig. 9) is parallel to the one obtained with test animals, but clearly shifted to the left.

Potentiation of Pentobarbital Activity

Previous administration of DOPA produces a clear potentiation of the hypnotic activity of pentobarbital. This activity optimal at the dose of 400 mg./kg. decreases afterwards, until it practically disappears with large doses (Fig. 10). Previous administration of pargyline (150 mg./kg.) does not change the shape of the curve which is simply shifted to the left. Maximum potentiation of sleep is noticed with a dose of 50 mg./kg. of DOPA.

DISCUSSION

It is undeniable that DOPA used alone produces, as doses go, a diphasic action: decrease, and then increase of motor activity. This activity also exists with barbituric sleep: at first there is a potentiation by DOPA and then, as doses increase, this phenomenon disappears. Comparing dose-activity curves obtained with both tests shows an excellent concordance: DOPA potentiates sleep inasmuch as it has a greater decreasing action on motor activity; higher doses of DOPA potentiate sleep so much less as they are high, and have no action at very high doses. This diphasic action explains the discrepancies in literature, mentioned earlier.

Are there two different activities with two distinct origins or, on the contrary, is there only one same activity, increasing with doses and able of taking two different shapes ? The question is not irrelevant. In fact, when the doses of DOPA used produce the greatest decrease of motor activity, the animals do not seem at all tranquillized but, on the contrary, are permanently awake and overexcitable. One could admit that, in this case, an

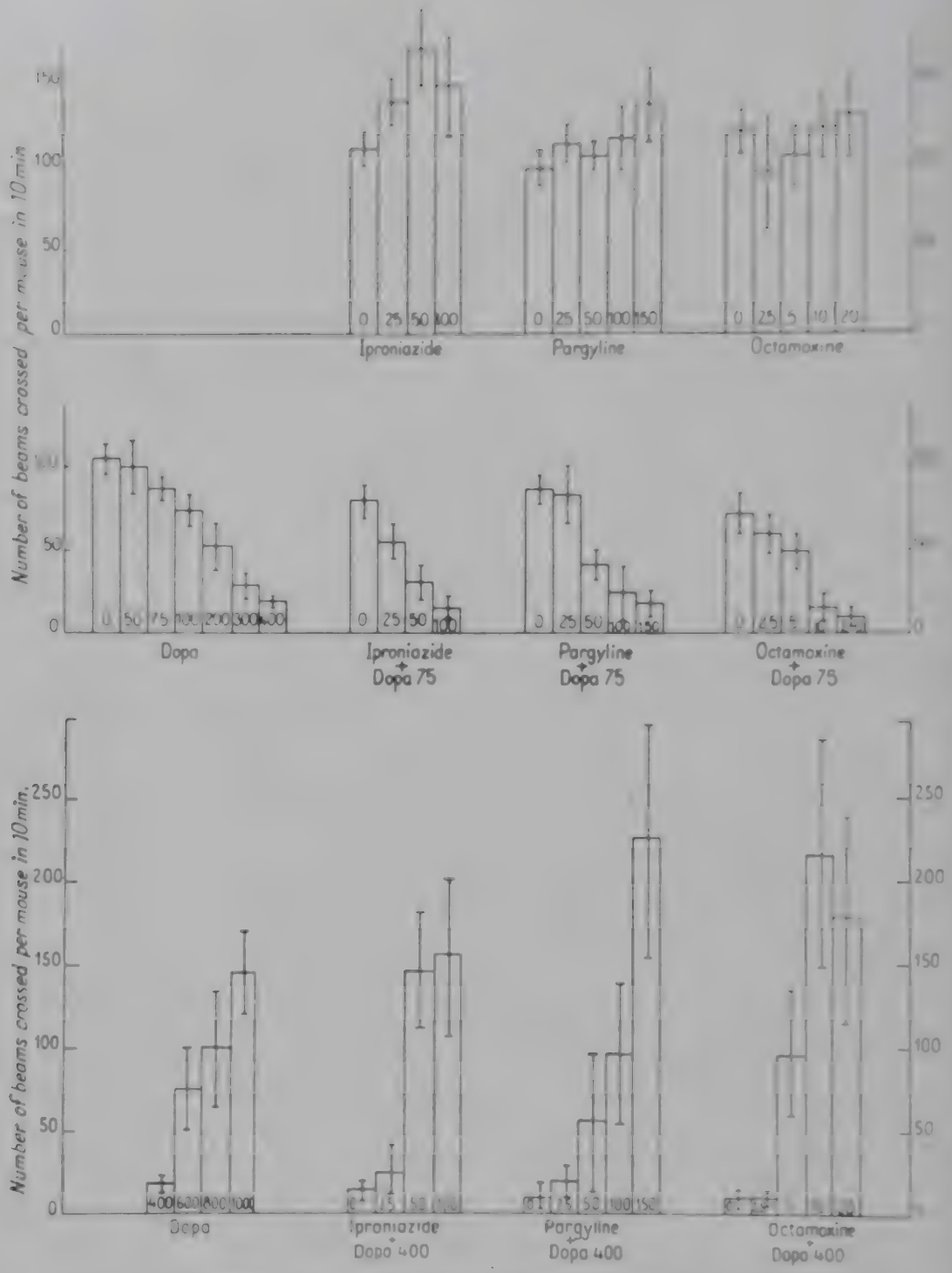

FIG. 8 — INFLUENCE OF MAOI PRETREATMENT ON DOPA TREATED OR NOT TREATED (HIGHER PART OF THE FIGURE): 75 mg./kg. (MIDDLE PART) OR 400 mg./kg. (LOWER PART) (Influence of varying doses of DOPA is shown as an indication on the left part)

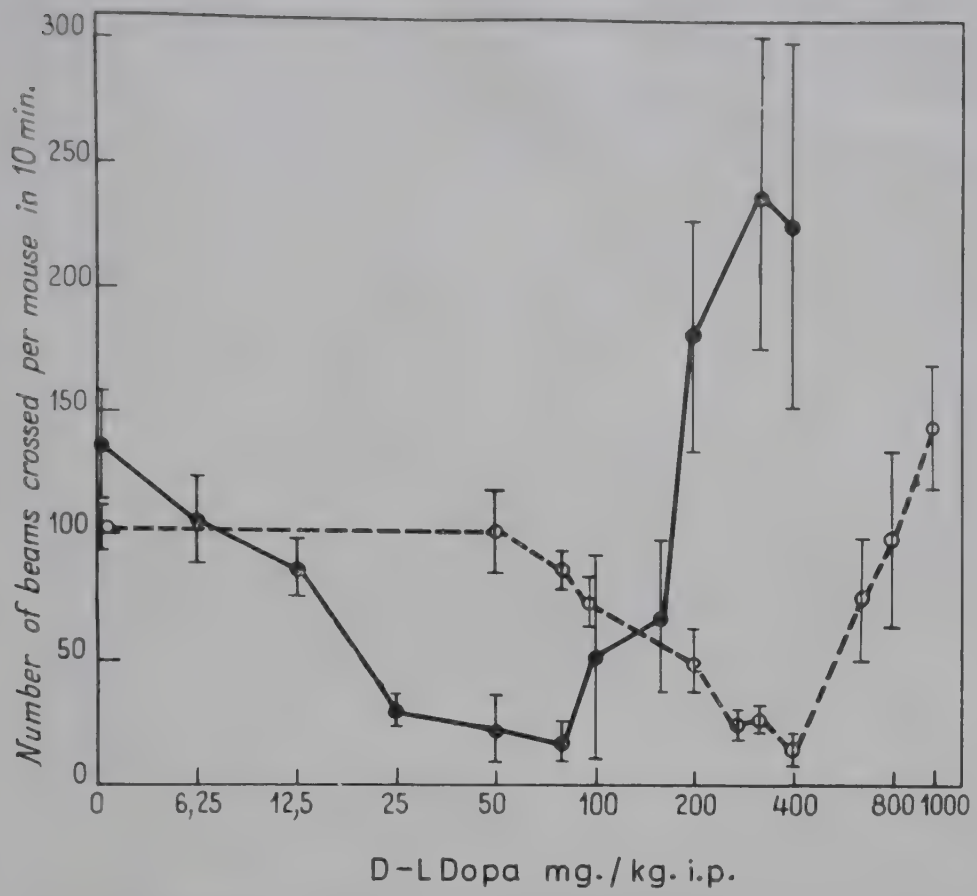

FIG. 9—INFLUENCE ON MICE MOTILITY OF DOPA, MAOI (PARGYLINE 150 mg./kg.) PRETREATED (FULL LINE) OR NOT (DOTTED LINE)

anxigeneous activity of DOPA could be enough to mask the motor excitation, as it was shown for amphetamine (Boissier, 1965; Boissier and Simon, 1965; Lat, 1965). Observation of the mice goes towards such an hypothesis.

Unluckily, it is difficult to admit that an anti-sleep activity accompanies an 'exciting' activity, and it seems as if there were two different activities.

A claim can be made for a biochemical hypothesis. Measurements of catecholamines in the brain after DOPA administration have shown a very fast accumulation of dopamine and noradrenaline (Bertler and Rosengren, 1962; Rosell *et al.*, 1963; Weiss, 1963; Wiegand and Perry, 1961). Doses of DOPA under 400 mg./kg. would be quickly transformed into dopamine, responsible for the decrease of motor activity, and then into noradrenaline, to excess of which sympathicotonia phenomenon would be due. Doses of DOPA above 400 mg./kg. could not be entirely transformed into dopamine by DOPA-decarboxylase, and DOPA's own motor stimulating activity would then come into evidence, the sympathicotonia phenomenon being explained as previously. No experimental reason allows at present to support this hypothesis. It would be important to know the activity of DOPA after specific blocking of DOPA-decarboxylase and/or β-hydroxylase.

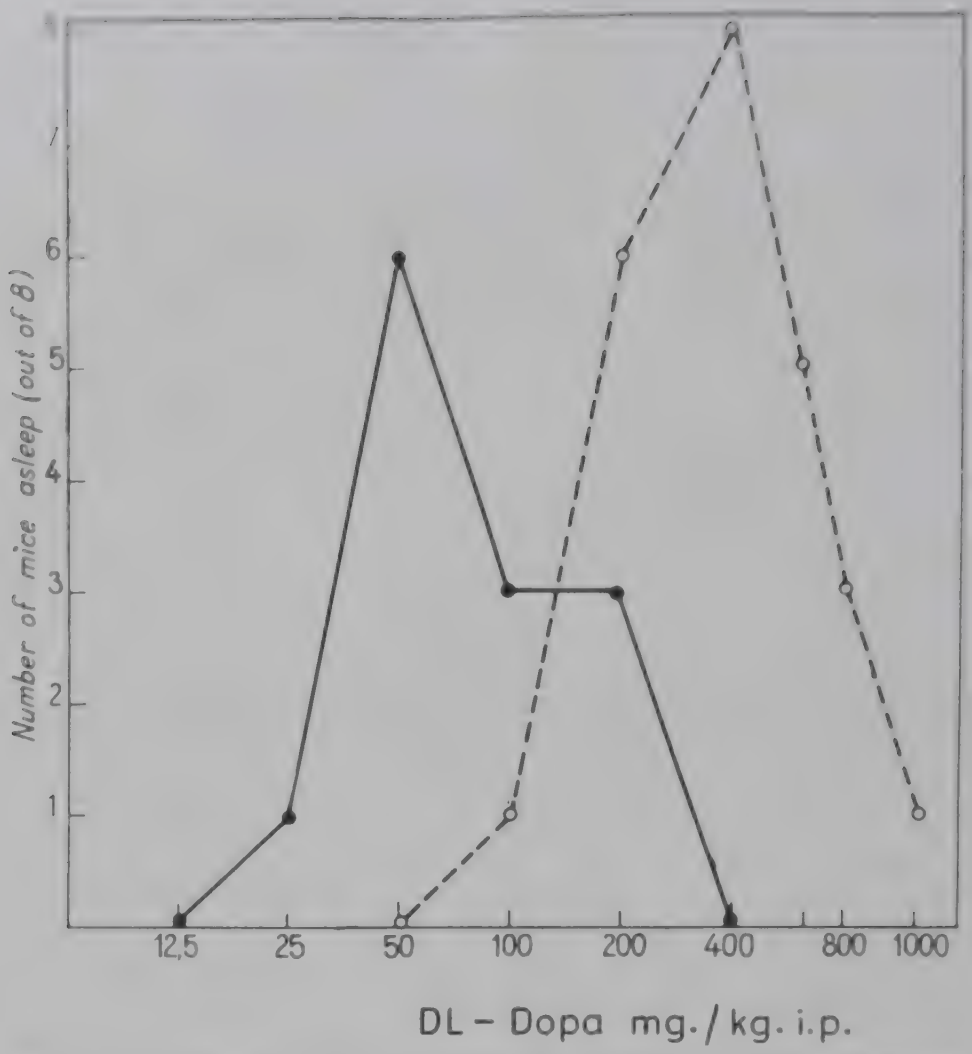

FIG. 10 — INFLUENCE IN MICE OF DOPA ON ACTIVITY OF AN INFRAHYPNOTIC DOSE OF PENTOBARBITAL IN MAOI (PARGYLINE 150 mg./kg.) PRETREATED (FULL LINE) OR NOT (DOTTED LINE)

MAOI produce a potentiation of DOPA, whether one considers action on motor activity or action on barbituric sleep; it is a fact that both curves are shifted to the left in a fairly homothetic way. MAO inhibition is enough to explain this potentiation phenomenon in the limits of the above hypothesis. However, it seems essential, when studying the potentiation of a dose of DOPA by a MAOI, to know precisely where this dose stands on the dose-activity curve of the test being used. Doses of DOPA used by most authors until now were in between 200 and 400 mg. kg., doses which produce a decrease of activity and whose potentiation by a MAOI gives an unquestionable rise in activity associated with a high lethality. It would seem more logical to choose a dose of DOPA of 75 mg. kg. which produces little or no variation in motor activity, but whose potentiation would give a collapse of this activity.

REFERENCES

1. Bertler, A. & Rosengren, E., *Experientia*, **15** (1962), 382.
2. Besendorf, H. & Pletscher, A., *Helv. physiol. pharmacol. Acta.*, **14** (1956), 383.
3. Boissier, J. R., *Proc. Second International Pharmac. Meeting*, Vol. 1, Pergamon Press (1965), 25.
4. Boissier, J. R., *Therapie*, **20** (1965), 1089.
5. Boissier, J. R. & Simon, P., *Arch. int. Pharmacodyn.*, **147** (1964), 372.
6. Boissier, J. R. & Simon, P., *Arch. int. Pharmacodyn.*, (1965) (in press).
7. Boissier, J. R. & Simon, P., *Psychopharmacologia*, (1965) (in press).
8. Brodie, B. B., Pletscher, A. & Shore, P. A., *J. Pharmac. exp. Ther.*, **116** (1956), 9.
9. Chessin, M., Kramer, E. R. & Scott, C. C., *J. Pharmac. exp. Ther.*, **119** (1957), 453.
10. Dews, P. B., *Br. J. Pharmac.*, **8** (1953), 46.
11. Everett, G. M., Davin, J. C. & Toman, J. E. P., *Fed. Proc.*, **18** (1959), 388.
12. Gylys, J. A., Muccia, P. M. R. & Taylor, M. K., *Ann. N. Y. Acad. Sci.*, **107** (1963), 899.
13. Lat, J., *Proc. Second International Pharmac. Meeting*, Vol. 1., Pergamon Press (1965), 47.
14. Rosell, S., Sedvall G. & Ullberg, S., *Biochem. Pharmacol.*, **12** (1963), 265.
15. Simon, P., *Therapie*, **20** (1965), 1123.
16. Smith, C. B. & Dews, P. B., *Psychopharmac.*, **3** (1962), 55.
17. Weiss, B., *Fed. Proc.*, **22** (1963), 540.
18. Wiegand, R. G. & Perry, J. E., *Biochem. Pharmac.*, **7** (1961), 181.

Studies Towards the Elucidation of Active Centres of Monoamineoxidase

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The rational use of monoamineoxidase inhibitors in the treatment of psychic depressions and other conditions including angina pectoris is seriously limited due to lack of knowledge regarding their mechanism of action. The enzyme monoamineoxidase has been shown, in recent years, to play an important role in adrenergic mechanisms and is responsible for the biological inactivation of 5-hydroxytryptamine (serotonin) and other sympathomimetic amines. Monoamineoxidase has frequently been suggested to be of biological importance in regulating the action of such substances[1,2] but such a function has not yet been clearly demonstrated in any instance. This enzyme oxidatively deaminates many compounds having amino groups attached to the terminal carbon atom, including 5-hydroxytryptamine and a number of sympathomimetic amines. At present inhibitors of this enzyme can be broadly classified into short-acting and long-acting inhibitors depending upon their reversible and irreversible properties. The increase in the 5-hydroxytryptamine and noradrenaline content of brain by the administration of long-acting hydrazine derivatives including iproniazid (Marsilid) and pheniprazine (JB 516, Catron) lasts for several days[3] indicating their prolonged action. Amphetamine and harmaline, on the other hand, cause an increase in the content of these amines in brain[4] which, however, returns to normal level after a few hours indicating their rapidly reversible nature of inactivation. The mode and exact mechanism of action of these inhibitors are still unknown. We have previously reported the nature of their enzyme inhibitory properties and

have compared these inhibitors with the well-known cholinesterase inhibitors[5]. It was found that *in vitro* these hydrazine derivatives including iproniazid and pheniprazine react relatively irreversibly with the active site(s) on the enzyme monoamineoxidase to produce a 'nonequilibrium antagonism'. Amphetamine, on the other hand, showed rapidly reversible competitive inhibition. The role of norepinephrine as a neurohumoral transmitting agent in the autonomic nervous system[6,7,8] has necessitated the study on the nature of inhibition and the reactive surface of monoamineoxidase. In the present study interaction between the long-acting inhibitors and the short-acting stereoisomeric amphetamines and two amphetamine derivatives, P-1726 (*p*-trifluoromethyl amphetamine) and P-1882 (*p*-S-methyl amphetamine) was investigated on the monoamineoxidase activity of the isolated rat liver mitochondria. It was found that amphetamine and P-1882 in concentrations low enough to have no effect on the enzyme activity were able to protect monoamineoxidase against inhibition by other long-acting inhibitors. With respect to both direct inhibition and protective ability against the other inhibitors, D-amphetamine was found to be more active than the corresponding L-isomer. In continuation of this work on the elucidation of the active centres of the enzyme we have observed that peanut oil inhibits the monoamineoxidase activity of the isolated rat liver mitochondria. Also, an unsaturated fatty acid, oleic acid, the chief constituent of peanut oil (Peanut oil contains: oleic acid, 60·6%; linoleic acid, 21·6%; stearic acid, 4·9%; palmitic acid, 6·3% and arachidic acid, 3·3%) showed all the properties of peanut oil in inhibiting the enzyme monoamineoxidase. Linoleic acid, another unsaturated fatty acid constituent of peanut oil, was also found to have similar monoamineoxidase inhibitory property, being equally potent as oleic acid. Unsaturation has been shown to be a prime factor in the enzyme inhibition since stearic acid, the saturated homologue of these acids, was found to have comparatively no effect on the enzyme activity[9]. During the course of these investigations an interesting effect of peanut oil and oleic acid was observed. Peanut oil and oleic acid were found to possess properties like amphetamine and amphetamine derivative, P-1882 in protecting the inhibition caused by pheniprazine, the nonequilibrium monoamineoxidase inhibitor[5,9].

MATERIALS AND METHODS

Adult rats weighing 200–250 g. were killed by a blow on the head followed by decapitation. The livers were homogenized in ice-cold 0·25 M. sucrose to give 10 per cent (wt/vol.) final concentration of the homogenate. Differential centrifugation of the homogenate by the method of Hogeboom *et al.*[10] as modified by Hawkins[11] was used for the isolation of mitochondria. These particles were suspended in sucrose so that 15 ml. of the suspension was equivalent to 10 g. of fresh liver after washing three times with 0·25 M. cold sucrose solution.

Determination of Monoamineoxidase Activity

Warburg manometric method. The oxygen uptake during oxidation of tyramine (0·01 M.), L-norepinephrine (0·01 M.) and 5-hydroxytryptamine (0·01 M.) by isolated rat liver mitochondria was used as an index of the enzyme activity[13]. Amphetamine, amphetamine derivatives, peanut oil and oleic acid were added to the main vessel with the enzyme preparation in accordance with the requirement of the experiment. The other long-acting monoamineoxidase inhibitors were added from the side arm 10–20 min. prior to the addition of the substrate which was also added from the second side arm.

Tyramine utilization. The oxidative deamination of tyramine was measured by the determination of the amount of tyramine utilized during the experimental period in order to see if the oxygen uptake in our experiments reflected the true enzyme activity. After the desired experimental period, 5 ml. of 10% trichloroacetic acid was added to each vessel and the precipitated proteins were removed by centrifugation. Suitable aliquots of the supernatant fluid were assayed for tyramine fluorimetrically with the help of Aminco-Bowman Spectrophotofluorometer. Tyramine gave maximum fluorescence at 310 mμ with an activating frequency of 275 mμ. In our experiments recoveries of tyramine added to mitochondrial preparations without incubation were quantitative.

Dialysis experiments. In these experiments 4·0 ml. of the mitochondrial suspension, in a total volume of 8·0 ml., was incubated at 37° C. in test tubes. Stereoisomeric amphetamines, P-1882, peanut oil and oleic acid were incubated with the enzyme preparation (mitochondrial suspension) for 15 min. in two sets of test tubes. In one set of these test tubes other long-acting monoamineoxidase inhibitors were added and all the tubes were incubated for an additional period of 30 min. After a total incubation period of 45 min. 3 ml. aliquots from the total of 8 ml. were dialysed against 1000 ml. of distilled water for varying lengths of time. The dialysed material was made up to a total volume of 8 ml. and 2 ml. aliquots were tested in triplicate for their monoamineoxidase activity.

In vivo experiments. Adult rats weighing 200–250 g. were fed 2 ml. of peanut oil daily for three days and sacrificed on the fourth day. Pheniprazine at a dose of 5 mg./kg. was administered intraperitoneally to normal as well as peanut oil-fed rats 30 min. before these animals were sacrificed. The monoamineoxidase activity of the liver and brain homogenates obtained from both normal and peanut oil-fed animals was determined manometrically according to the method described by Creasy[13]. The mitochondria were isolated from the liver obtained from normal and peanut oil-fed animals. Monoamineoxidase activity of these isolated mitochondria was determined using tyramine as the substrate in the presence and in the absence of 1×10^{-6} M. pheniprazine.

TABLE 1—INHIBITION OF MONOAMINEOXIDASE BY STEREOISOMERIC AMPHETAMINES

D-Amphetamine and L-amphetamine were used at a final concentration of 3 10 ^{3}M. Details of the assay procedure and vessels contents are described in the text.)

Additions	Enzyme activity/60 min./500 mg.			
	Oxygen uptake *μ moles*	% Inhibition by amphetamine	Tyramine utilized *μ moles*	% Inhibition by amphetamine
Control	18·42	..	18·60	..
	21·42	..	21·72	..
	18·54	..	18·03	..
D-Amphetamine	9·99	45·57	9·60	48·39
	10·02	53·22 (50·2)	10·48	51·75
	8·97	51·62	9·30	48·42
L-Amphetamine	11·46	37·79	12·00	35·48
	13·14	38·66 (37·08)	13·46	38·03
	12·09	34·79	12·04	32·31

RESULTS

The results of the study of the inhibition of monoamine oxidase by the two enantiomorphs of amphetamine is shown in Table 1. It was found that D-amphetamine caused greater inhibition of the enzyme activity compared to the L-isomer under similar experimental conditions. Inhibitory effects of D-amphetamine were found to be similar when the enzyme activity was determined by the tyramine utilization method. Our results as indicated in Table 1 show the stoichiometric relationship between oxygen uptake and tyramine utilization where one micromole of tyramine was found to be oxidized for every micromole of the oxygen utilized in these experiments. Our results are in good agreement with those of Pratesi and Blaschko[14] who have reported that the amineoxidase of guinea-pig liver was inhibited to a greater extent by the dextrorotatory isomer of amphetamine than by levorotatory isomer. Recently, Blaschko and Stromblad[15] have also reported that pharmacologically more active D-amphetamine is a stronger inhibitor of monoamineoxidase of the human brain, and of the human parotid and submaxillary glands. The inhibitory pattern of D-amphetamine and L-amphetamine was similar during oxidative deamination of L-norepinephrine and 5-hydroxytryptamine. Pre-incubation of the enzyme preparation with either of the isomers did not alter their enzyme inhibitory properties. These results indicated that a rapid equilibrium existed between amphetamines with the substrate and also that the enzyme inhibitor complex is readily dissociated.

The nature of the inhibition by the two isomers of amphetamine as evaluated by means of the conventional graphic method of Lineweaver

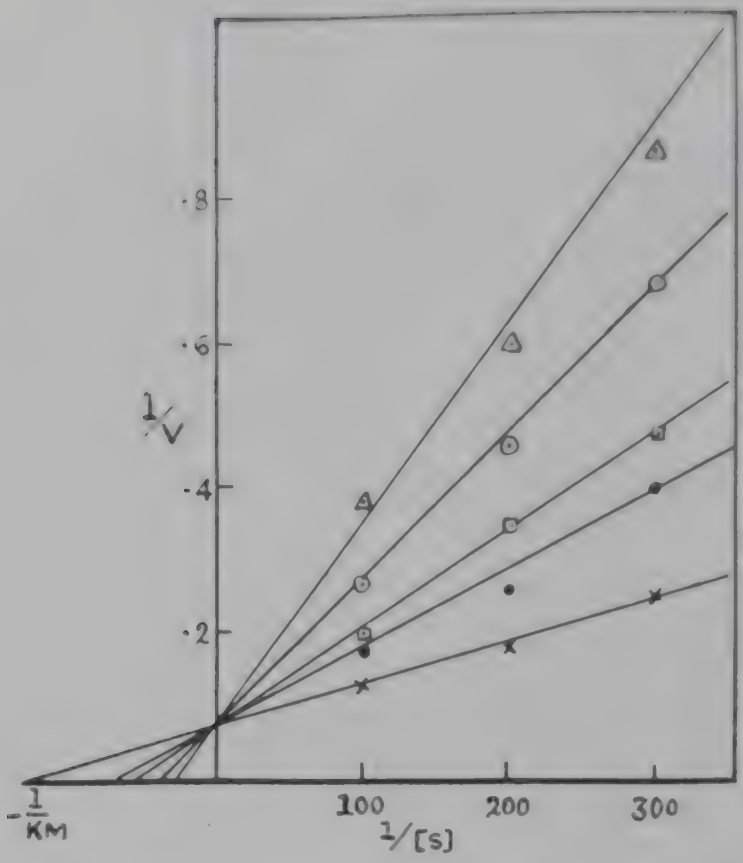

FIG. 1 — KINETIC STUDY OF SUBSTRATE-COMPETITIVE INHIBITION BY D-AMPHETAMINE AND L-AMPHETAMINE OF RAT LIVER MITOCHONDRIAL MONOAMINEOXIDASE DERIVED FROM 333 mg. OF WET TISSUE [1/V, Reciprocal × 10 μl. oxygen consumed per 10 min.; S, molar concentration of tyramine; X—X, control; ⊡—⊡, D-amphetamine (0·001 M.); △—△, D-amphetamine (0·003 M.); ●—●, L-amphetamine (0·001 M.); ⊙—⊙, L-amphetamine (0·003 M.)]

and Burk[16] is shown in Fig. 1. The intercept at 1/S axis was taken as $-1/K_m$; and the value of 8×10^{-3} M. obtained as the Michaelis constant (K_m) for the enzyme preparation was found to be identical with the one reported earlier[17]. Both isomers were found to be competitive inhibitors of monoamine-oxidase. In plots of 1/V with respect to inhibitor concentration (I) at different substrate concentrations, the intercept is at the point where $I = K_i$[18]. The K_i (dissociation constant) values were determined graphically and were found to be $8{\cdot}5 \times 10^{-4}$ M. for D-amphetamine and $1{\cdot}4 \times 10^{-3}$ M. for L-amphetamine.

The inhibitory effects of pheniprazine on the monoamineoxidase activity and the protection afforded by D-amphetamine from the inhibition produced by pheniprazine are represented in Table 2. D-amphetamine at a concentration of 3×10^{-5} M. was found to have no effect on the oxygen uptake as well as on the amount of tyramine utilized indicating that D-amphetamine at these concentrations has no effect on the monoamine-oxidase activity. Pheniprazine (β-phenyl isopropyl hydrazine) which has

TABLE 2—PROTECTION OF PHENIPRAZINE INDUCED INHIBITION OF MONOAMINEOXIDASE BY D-AMPHETAMINE

[Assay procedure and vessel contents are described in the text. D-Amphetamine (3×10^{-5}M.) was initially present in the vessel with the enzyme preparation. Pheniprazine was added 15 min. prior to the addition of tyramine (1×10^{-2}M.). Values in molar concentration represent final concentration of the inhibitors.]

Additions	Enzyme Activity/60 min./500 mg.			
	Oxygen uptake *μ moles*	Inhibition	Tyramine utilized, *μ moles*	% Inhibition
Control	16·97	..	17·56	..
	18·51	..	18·40	..
	17·68	..	18·00	..
D-Amphetamine (3×10^{-5} M.)	16·97	..	17·56	..
	18·82	..	18·44	..
	17·59	..	18·08	..
Pheniprazine (1×10^{-6} M.)	6·25	63·17	5·85	66·69
	6·83	63·10 (62·72)	6·86	62·72
	6·74	61·88	6·76	62·45
D-Amphetamine (3×10^{-5} M.) + Pheniprazine (1×10^{-6} M.)	9·69	42·90	10·24	41·69
	10·02	46·76 (44·16)	10·46	43·28
	10·06	42·81	9·88	45·35

been shown to possess marked enzyme inhibitory activity[19] was found to inhibit the oxygen uptake by 62·72 per cent at a final concentration of 1×10^{-6} M. Similarly tyramine utilization was inhibited to the extent of 63·95 per cent by pheniprazine under identical experimental conditions. As is evident from Table 2, concentrations of D-amphetamine which had no effect on the enzyme activity were yet able to protect monoamine-oxidase against the inhibition caused by pheniprazine. Such protection was observed only when D-amphetamine was incubated with the enzyme preparation prior to the addition of pheniprazine or when both D-amphetamine and pheniprazine were added together. Addition of D-amphetamine to the enzyme preparation after pheniprazine was found to have no protective effect. The degree of protection observed by D-amphetamine as measured by the increase in oxygen uptake was equivalent to that of the tyramine utilized in these experiments.

The ability of stereoisomeric amphetamines to protect the enzyme was investigated against the inhibition by other well-known monoamine-oxidase inhibitors, i.e. iproniazid[20], pheniprazine[19], tranylcypromine[21], nialamide[17] and P-1726[17]. It was found that the degree of protection afforded by D-amphetamine was greater than the corresponding L-isomer (Table 3). Both D- and L-amphetamines were found to have no effect on the enzyme activity at a final concentration of 3×10^{-5}M.

TABLE 3—STEREOSPECIFICITY OF AMPHETAMINE DURING PROTECTION AGAINST MONOAMINEOXIDASE INHIBITION IN ISOLATED RAT LIVER MITOCHONDRIA

(Details of the assay procedure and vessel contents are described in the text. Inhibition was calculated on the basis of decrease in oxygen uptake during 45 min from rat liver mitochondria derived from 385 mg. wet tissue. Amphetamine where added was initially present in the main vessel together with mitochondrial preparation. Tranylcypromine and nialamide were added 20 min and pheniprazine, tranylcypromine and P-1726 were added 15 min before adding tyramine. Values in molar concentration represent final concentration of the inhibitors.)

Inhibitors	% Inhibition		
	Amphetamine absent	Amphetamine present (3×10^{-4} M.)	
		D-Amphetamine	L-Amphetamine
Iproniazid (1×10^{-5} M.)	51·2	32·0	40·0
	50·3	30·8	42·0
	48·8	30·6	39·8
	54·0	33·0	40·2
Pheniprazine (2×10^{-6} M.)	85·5	57·2	63·1
	84·3	56·8	63·4
	86·0	55·9	64·0
	83·7	58·0	63·8
Tranylcypromine (6×10^{-6} M.)	73·8	57·2	63·1
	74·0	56·8	63·4
	76·6	55·9	64·0
	71.3	58·0	63·8
Nialamide (1×10^{-4} M.)	61·7	50·7	60·0
	61·0	49·8	60·8
	63·4	51·3	60·7
	62·0	50·5	59·8
P-1726 (6×10^{-6} M.)	68·4	55·4	61·3
	70·0	57·7	62·8
	71·2	57·6	64·3
	70·4	58·1	63·3

Dialysis experiments using high inhibitory concentrations (3×10^{-4} M.) of D-amphetamine and L-amphetamine were conducted in an attempt to investigate if the greater protective ability of D-amphetamine compared to the L-isomer is due to its greater affinity for the enzyme monoamine-oxidase as shown by its being a stronger inhibitor or a stereoisomeric effect. The experiments were conducted according to the method described in the text and the results of these dialysis experiments are presented in Table 4. These experiments indicating reversible and irreversible nature of these monoamineoxidase inhibitors are in good agreement with studies dealing with evaluation of their enzyme inhibition[17]. In the present study, complete restoration of the enzyme activity was observed on dialysis of the enzyme preparation incubated with either of the stereoisomers of amphetamine.

TABLE 4—DIALYSIS EXPERIMENTS SHOWING GREATER PROTECTION OF MONOAMINEOXIDASE INHIBITION BY D-AMPHETAMINE

[Details of the assay procedure and vessel contents are described in the text. Mitochondrial suspensions (4 ml.) in a total volume of 8 ml. were incubated with D- and L-amphetamine at a final concentration of 3×10^{-3} M. for 15 min. prior to the addition of other monoamine oxidase inhibitors. After a total incubation period of 45 min. 3 ml. aliquots were dialysed against 1000 ml. of distilled water for 16-24 hr. Dialysed preparations were made up to the total volume of 8 ml. and 2 ml. aliquots were tested for enzyme activity. Values in molar concentration represent final concentration of the inhibitors.]

Additions	Enzyme Activity, μMO_2/45 min./ 500mg.		
	Amphetamine absent	Amphetamine present	
		D-Amphetamine	L-Amphetamine
Control	12·30	14·74	14·96
	12·06	13·68	12·92
	12·62	13·15	12·80
	14·64	17·77	17·08
Iproniazid (3×10^{-5} M.)	4·73	8·30	6·88
	4·88	8·64	6·54
	4·59	8·98	5·96
	4·36	8·48	6·38
Pheniprazine (3×10^{-6} M.)	1·38	8·00	5·72
	1·25	8·81	7·26
	1·28	8·82	7·56
	1·14	7·88	6·36
Tranylcypromine (3×10^{-6} M.)	2·40	7·23	4·11
	2·95	7·85	4·22
	3·06	6·48	4·39
	2·36	7·06	4·08
Nialamide (3×10^{-5} M.)	4·73	9·25	7·20
	4·88	9·06	6·86
	5·12	8·99	7·03
	4·06	9·12	7·12

D-Amphetamine was found to afford greater protection against the inhibition caused by other inhibitors in such dialysis experiments also as compared with L-amphetamine. The ability of L-amphetamine to protect the enzyme against the inhibition by nialamide was established in such dialysis experiments which, however, was not well marked in other studies (Table 3). Similar results with dialysis experiments were also obtained where the enzyme activity was measured by estimating the amount of tyramine utilized under identical experimental conditions.

Enzyme activity in dialysis experiments was also determined using 5-hydroxytryptamine as the substrate in an attempt to investigate if the ability of stereoisomeric amphetamines to protect from inhibition by other

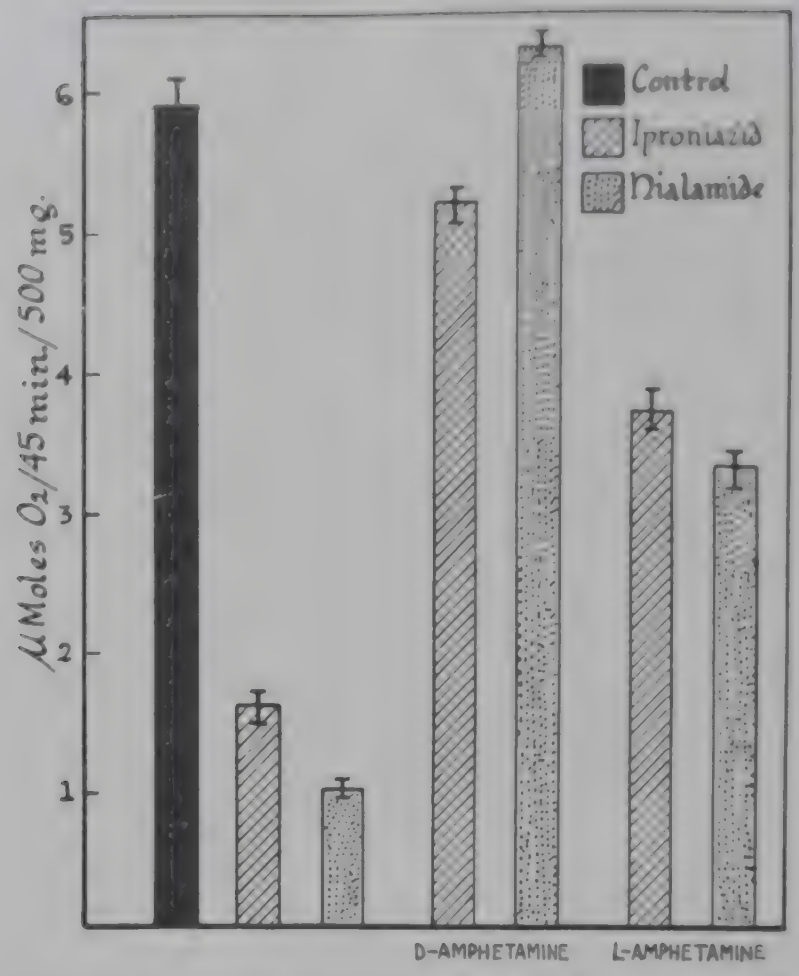

FIG. 2 — DIALYSIS EXPERIMENTS SHOWING GREATER ABILITY OF D-AMPHETAMINE TO AFFORD PROTECTION AGAINST INHIBITION OF MONOAMINEOXIDASE BY IPRONIAZID AND NIALAMIDE DURING OXIDATIVE DEAMINATION OF 5-HYDROXYTRYPTAMINE

inhibitors could be demonstrated with substrates other than tyramine. As is indicated in Fig. 2, D-amphetamine was found to afford greater protection than L-amphetamine against the inhibition caused by iproniazid and nialamide during oxidative deamination of 5-hydroxytryptamine. The enzyme activity with serotonin was approximately 2/3 as compared with that observed with tyramine. In other studies, no such protection could be observed during interaction of pheniprazine, a potent irreversible inhibitor, with other moderately reversible inhibitors like iproniazid or nialamide. These results indicate that the ability of amphetamine to afford protection from the monoamineoxidase inhibition is not due to its reversible nature of inactivation[17].

Furthermore, studies were conducted with two amphetamine derivatives P-1882 and P-1726 to see if the protective ability of amphetamine could be observed with these amphetamine derivatives. The results of the interaction between P-1882a nd P-1726 and other long-acting monoamineoxidase inhibitors are shown in Table 5. It was found that concentrations of P-1882

TABLE 5 INTERACTION BETWEEN MONOAMINEOXIDASE INHIBITORS AND AMPHETAMINE DERIVATIVES

(Details of the assay procedure and vessel contents are described in the text. Amphetamine derivatives where added were initially present in the main vessel together with mitochondrial preparation. Inhibition was calculated on the basis of the decrease in oxygen uptake during 45 min. from rat liver mitochondria derived from 333 mg. wet tissue. Pheniprazine, iproniazid and tranylcypromine were added 10 min. prior to the addition of tyramine. Values in molar concentration represent final concentration of the inhibitors.)

Inhibitor	Control	Inhibition, %	
		P-1882 (1×10^{-5} M.)	P-1726 (1×10^{-6} M.)
Iproniazid (3×10^{-5} M.)	80·9	49·8	81·2
	82·8	49·6	80·6
	81·6	51·2	80·9
	82·2	50·4	81·3
Pheniprazine (1×10^{-6} M.)	46·1	31·0	46·2
	43·8	29·3	45·8
	44·0	30·4	44·9
	45·3	29·4	46·3
Pheniprazine (3×10^{-6} M.)	91·2	58·9	92·8
	89·9	59·8	93·0
	90·8	61·0	90·8
	91·4	61·6	92·6
Tranylcypromine (1×10^{-5} M.)	94·8	78·6	95·8
	95·2	79·2	95·1
	95·1	82·0	96·2
	93·9	79·1	94·9

having no effect on the enzyme activity were able to protect the enzyme from the inhibition caused by pheniprazine, iproniazid and tranylcypromine. Such protection, like the one observed with amphetamine, was only observed when P-1882 was incubated with the enzyme preparation prior to the addition of other inhibitors or when both these inhibitors were added together. The other amphetamine derivative P-1726 was devoid of any such protective ability and was found to be a more potent and persistent inhibitor of monoamineoxidase than either amphetamine or P-1882[17]. However, D- and L-amphetamines were able to afford protection from the inhibition caused by P-1726 (Table 3) where greater protection was observed with D-amphetamine. Dialysis experiments have also indicated the ability of P-1882 to afford protection against the inhibition caused by pheniprazine.

In vivo administration of peanut oil was found to have no effect on the monoamineoxidase activity of the liver and the brain homogenate (Table 6). Administration of pheniprazine (5 mg./kg.) caused definite inhibition of

TABLE 6—*IN VIVO* EFFECT OF PEANUT OIL ON MONOAMINEOXIDASE ACTIVITY OF ISOLATED LIVER AND BRAIN HOMOGENATES

[The animals were fed 2 ml. peanut oil daily for three days and were sacrificed on the fourth day. Pheniprazine (5 mg./kg.) was administered intraperitoneally to control and peanut oil-fed rats 30 min. before they were sacrificed. The vessel contents and assay procedure are as indicated in the text. Tyramine was used as the substrate.]

Experiments	$\mu l.O_2$ 60 min./500 mg.	
	Liver	Brain
Control	305	206
Control – Pheniprazine	193	93
Control – Peanut oil	342	213
Control – Peanut oil + Pheniprazine	204	168

TABLE 7—*IN VITRO* EFFECT OF PHENIPRAZINE ON ISOLATED LIVER MITOCHONDRIA FROM CONTROL AND PEANUT OIL-FED RATS

[The vessel contents and assay procedure are as indicated in the text. Pheniprazine (1×10^{-6} M.) was added to isolated mitochondrial preparations 5 min. prior to the addition of tyramine.]

Experiments	$\mu l.O_2$/60 min./500 mg.	
	—Pheniprazine	+ Pheniprazine
Control	234	106 (55)
Peanut oil-fed rat	256	182 (26)

Figures in parentheses represent per cent inhibition

monoamineoxidase activity in both liver and brain homogenates. The degree of inhibition due to administration of pheniprazine in animals fed with peanut oil was found to be less with the brain homogenate. However, no definite protection could be observed with liver homogenate in these experiments. On the other hand, the ability of peanut oil to afford protection against inhibition by pheniprazine was observed in *in vitro* experiments using isolated liver mitochondria from both normal and peanut oil-fed rats. As is evident from Table 7, the inhibition of monoamineoxidase by pheniprazine (1×10^{-6} M.) was 55 per cent in liver mitochondria obtained from normal animals. The inhibition, on the other hand, was only 23 per cent with liver mitochondrial preparation obtained from animals which were fed with peanut oil for three days.

The ability of peanut oil and oleic acid to protect the enzyme monoamineoxidase against the inhibitory effects of iproniazid, pheniprazine and

TABLE 8—*IN VITRO* EFFECT OF PEANUT OIL AND OLEIC ACID ON INHIBITION OF MONOAMINEOXIDASE

[Assay procedure and vessel contents are described in the text. Peanut oil and oleic acid were initially present in the vessel with the enzyme preparation. Pheniprazine, tranylcypromine and iproniazid were added 15 min. prior to the addition of tyramine (1×10^{-2} M.)]

Additions	Control	% Inhibition	
		Peanut oil	Oleic acid
Pheniprazine	91	72	66
Tranylcypromine	75	62	59
Iproniazid	85	74	67

TABLE 9—PROTECTIVE EFFECT OF VARYING CONCENTRATIONS OF OLEIC ACID ON MONOAMINEOXIDASE

(The enzyme activity of isolated rat liver mitochondria is represented as oxygen uptake where figures marked with an asterisk indicate the amount of tyramine utilized in these experiments. The vessel contents and assay procedure are indicated in the text.)

Additions	μMoles/60 min./250 mg.		
	— Oleic acid	+ Oleic acid	
		$3{\cdot}3 \times 10^{-3}$ M.	$6{\cdot}6 \times 10^{-3}$ M.
None	13·4 12·9*	9·6 (28) 9·4* (27)	6·2 (54) 6·3* (51)
Pheniprazine	2·0 (85) 1·9* (85)	5·0 (48) 4·9* (48)	4·8 (22) 4·6* (27)

Figures in parentheses represent per cent inhibition

tranylcypromine is well demonstrated in Table 8. As is evident from this Table, definite decrease in the per cent inhibition was observed when the enzyme preparations were incubated with either peanut oil or oleic acid prior to the addition of the long-acting monoamineoxidase inhibitors. The concentrations of peanut oil or oleic acid used in these experiments were devoid of any inhibitory effect on the enzyme activity.

Furthermore, effect of varying concentrations of oleic acid was investigated on the monoamineoxidase activity of rat liver mitochondria. It was found that inhibitory concentrations of oleic acid ($3{\cdot}3 \times 10^{-3}$ M., $6{\cdot}6 \times 10^{-3}$ M.) were also able to afford protection against the inhibition caused by pheniprazine. Such a protective effect of oleic acid is presented in Table 9, where protection was observed when the enzyme activity was determined as the oxygen uptake and as well as with tyramine utilization.

TABLE 10—DIALYSIS EXPERIMENTS SHOWING PROTECTION OF PHENIPRAZINE INHIBITION BY OLEIC ACID

(The vessel contents and assay procedure are as indicated in the text. Oleic acid was used at a final concentration of 1×10^{-3} M. Tyramine was used as the substrate.)

Additions	μlO_2 /60 min./250 mg.	
	Undialysed	Dialysed
Control	316	261
Oleic acid	327	261
Control + Pheniprazine	202 (36)	150 (43)
Control + Oleic acid + Pheniprazine	243 (25)	212 (19)

Figures in parentheses represent per cent inhibition

The ability of oleic acid to afford protection against inhibition by pheniprazine was also demonstrated in dialysis experiments (Table 10). The degree of protection by oleic acid observed in undialysed preparation was further increased on dialysis. Oleic acid used at a final concentration of 0·01 M. was found to have no effect on the enzyme activity. Similar dialysis experiments using peanut oil, at concentrations having no effect on the enzyme activity, indicated protection of the inhibition produced by pheniprazine.

Finally, kinetic studies have indicated that oleic acid, unlike amphetamine, was found to be a non-competitive inhibitor of monoamineoxidase[2].

DISCUSSION

Interaction between stereoisomeric amphetamines, amphetamine derivatives (P-1882, P-1726) and oleic acid was investigated in an attempt towards elucidation of the active centres of the enzyme monoamine oxidase. Various methods employed for measuring monoamineoxidase activity depend on either oxygen uptake[12,20] or amine disappearance[22]. In the present study standard manometric methods were employed where oxygen uptake indicated the enzyme activity during oxidative deamination of tyramine or 5-hydroxytryptamine. The amount of tyramine utilized in these experiments was estimated in order to see if the oxygen uptake represents the true activity of the enzyme. It was found that for the oxidation of 1 μ mole of tyramine equivalent amount of oxygen was consumed. The use of cyanide and semicarbazide used during the assay of monoamineoxidase[13] was, therefore, not necessary. Oxygen consumed in our experiments was found to reflect the true activity of the enzyme preparations.

The present study points the possibility of the existence of a close similarity in the site of action of stereoisomeric amphetamines, P-1882 and other monoamineoxidase inhibitors. Attachment of amphetamine and P-1882 could possibly be at similar active centres or 'receptor' sites which are particularly sensitive to the action of other monoamineoxidase inhibitors. As in the case of direct inhibition, D-amphetamine was more active than the L-isomer in protecting monoamineoxidase against other inhibitors. Thus it could be presumed that isomers of amphetamine and P-1882 block these active centres (receptors) on the enzyme molecule reversibly and thereby prevent other inhibitors from their attachment to these sites. The stereospecificity of the isomers of amphetamine and the presence of optically active centres of monoamineoxidase observed in the present study are in good agreement with those reported by other investigators[23]. Belleau *et al.*[24] have reported absolute stereospecificity of monoamineoxidase involved in adrenergic mechanism since configuration dependent deuterium isotope effect was observed during enzymic oxidation of asymmetrically labelled tyramine[24]. It seems that certain stereoisomeric factors are presumably involved during protection by two enantiomorphs of amphetamine. Dialysis experiments with higher concentrations of amphetamines provide additional evidence regarding the involvement of a stereoisomeric factor during protection of monoamineoxidase by two enantiomorphs of amphetamine. The ability of P-1882 alone to afford protection points towards the existence of primary and secondary site(s) on the enzyme molecule involved in the formation of enzyme-substrate or enzyme-inhibitor complex. The attachment of an inhibitor to the secondary centre, in addition to the primary essential centre represented by the amine moiety, could be responsible for greater inhibition and irreversible nature of inactivation. The non-competitive nature of inhibition and the protection of pheniprazine inhibition by oleic acid observed in these experiments indicate that pheniprazine and oleic acid are acting at different receptor sites on the enzyme monoamineoxidase. These results provide further evidence regarding the existence of two or more active centres on the enzyme molecule which are essentially involved in the formation of enzyme-substrate or enzyme-inhibitor complexes. Thus the interaction between nonequilibrium inhibitor (pheniprazine) and a non-competitive inhibitor (oleic acid), suggest that either these receptor sites are sufficiently closely related spatially to allow steric hindrance or that oleic acid in some way is concerned in changing the conformation of pheniprazine receptors[25]. The unsaturated groupings in the fatty acids appear to be critical for both direct inhibition and interference with the action of other monoamine inhibitors. Further studies dealing with the reactivation of *in vitro* blocked amineoxidase may give an insight of the possible role of feedback mechanisms in the course of the restoration of enzyme activity. At present hydrazide and hydrazine derivatives are being considered as potential drugs for

the treatment of depressed mental state. These drugs due to their irreversible nature have a disadvantage of persistence of action even after the drug is stopped. The protection of the inhibition due to long acting inhibitors by amphetamine and oleic acid may allow the therapeutic use of the combination of these drugs and thus may have considerable clinical value.

SUMMARY

Antagonism between stereoisomeric amphetamines, amphetamine derivatives and other monoamineoxidase (MAO) inhibitors has been investigated. MAO activity of isolated rat liver mitochondria was also measured by the estimation of tyramine spectrophotofluorometrically to show that the oxygen uptake reflects the true enzyme activity. Inhibition studies with MAO inhibitors have demonstrated a criterion for the nature of enzyme inhibition. Evidence has also been presented to show interrelationship between unsaturated fatty acids and MAO where oleic acid and linoleic acid were found to inhibit MAO. Stearic acid, on the other hand, was ineffective. Concentration of oleic acid, chief constituent of peanut oil, without affecting enzyme activity, protected MAO against inhibition by other long-acting MAO inhibitors. Similar protection against MAO inhibition was observed with peanut oil alone both *in vivo* and *in vitro* experiments. Kinetic studies indicated non-competitive inhibition of MAO by oleic acid. The unsaturated grouping in the fatty acid appears to be critical for both direct inhibition and interference with other MAO inhibitors. Such interactions suggest either that these active centres are sufficiently closely related spatially to allow steric hindrance or that oleic acid in some way changes the conformation of active centres responsible for the attachment of MAO inhibitors. The results point to the existence of primary and secondary centres on the enzyme molecule where two or more points of attachment of the inhibitor to the enzyme are required for full inactivation.

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REFERENCES

1. Zeller, E. A., *Pharmac. Rev.*, **11** (1959), 387.
2. Belleau, B., Adrenergic Mechanisms. A Ciba Foundation Symposium, ed. J. R. Vane, G. E. W. Wolstenholme and M. O'Cooner, J. & A. Churchill Ltd, London, (1960), 223.
3. Udenfriend, S., Weissbach, H. & Bogdanski, D. F., *Ann. N. Y. Acad. Sci.*, **66** (1957), 602.
4. Pletscher, A., *Experientia*, **12** (1956), 479.
5. Nickerson, M. & Parmar, S. S., *Fed. Proc.*, **20** (1961), 165.
6. Holtz, P., *Klin. Wschr.*, **28** (1950), 145.
7. Holtz, P., *Dtsch. Med. Wschr.*, **80** (1955), 2.
8. U. S. von Euler. Noradrenaline. Charles C. Thomas, Springfield, Illinois (1956).
9. Hollenberg, N. K., Parmar, S. S. & Nickerson, M., *Proc. Canad. Fed. Biol. Soc.*, **6** (1963), 30.
10. Hogeboom, G. H., Schneider, W. C. & Palade, G. E., *J. biol. Chem.*, **172** (1948), 619.
11. Hawkins, J., *Biochem. J.*, **50** (1952), 577.
12. Davidson, A. N., *Biochem. J.*, **67** (1957), 316.
13. Creasy, N. H., *Biochem. J.*, **64** (1956), 178.
14. Pratesi, P. & Blaschko, H., *Br. J. Pharmac.*, **14** (1959), 256.
15. Blaschko, H. & Stromblad, B. C. R., *Drug Research* (Arzneim Forsch), **10** (1960), 327.
16. Lineweaver, H. & Burk, D., *J. Am. chem. Soc.*, **56** (1934), 658.
17. Parmar, S. S., *Biochem. Pharmac.*, **15** (1966).
18. Hellerman, L., Reiss, O. K., Parmar, S. S., Wein, J. & Lasser, N. L., *J. biol. Chem.*, **258** (1960), 2468.
19. Horita, A., *J. Pharmac. exp. Ther.*, **122** (1958), 176.
20. Zeller, E. A., Barsky, J. & Berman, E. R., *J. biol. Chem.*, **214** (1955), 267.
21. Maass, A. R. & Nimmo, M. J., *Nature*, **184** (1959), 547.
22. Sjoerdina, A., Smith, T. E., Stevenson, T. D. & Udenfriend, S., *Proc. Soc. exp. Biol. Med.*, **89** (1951), 36.
23. Belleau, B., Burba, J., Pindell, M. & Reiffenstein, J., *Science*, **133** (1961), 102.
24. Belleau, B., Fang, M., Burba, J. & Morna, J., *J. Am. chem. Soc.*, **82** (1960), 5752.
25. Parmar, S. S., Hollenberg, N. K. & Nickerson, M. (in preparation).

A Study on the Properties of Purified Monoamineoxidase of Rat Brain Mitochondria

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Various neuropharmacological studies have stressed the importance of monoamineoxidase in the central nervous system and some of the important inhibitors of the enzyme have wide clinical uses. Monoamineoxidase activity in brain has been studied by various workers in crude tissue system[1-4]. Several attempts have been reported in literature to solubilize the mitochondria bound monoamineoxidase of animal tissues and purify them. These have met with limited success. A simple method based on lysis of mitochondria by ultrasonic waves followed by chromatography on DEAE cellulose column has been developed in this laboratory for solubilizing and purifying liver mitochondrial MAO[5]. The method has now been applied to rat brain. A preliminary report on some of the properties of a partially purified monoamineoxidase of rat brain mitochondria is presented here.

A soluble monoamineoxidase of rat brain mitochondria was prepared according to the method used for rat liver mitochondria[5] with some modifications. The mitochondrial suspension held in 0·01 M. phosphate buffer *p*H 7·6 was sonicated in a Mullard Magnetostricter at 25 kc./s. for 30 min. The supernatant obtained after centrifugation at 1,000,000 *g*. for 1 hr was dialysed and passed through a DEAE cellulose column previously equilibrated with 0·01 M. phosphate buffer *p*H 7·6 containing 0·01 M. NaCl. About 50–60 mg. of protein was applied to the column (1·2 × 12 cm.) and the elution was performed with increasing concentrations of NaCl. The first few fractions were found to be active, while a high amount of inactive protein emerged at a NaCl concentration of 0·5 M. The active fractions (Fig. 1) were pooled and usually had a specific activity of 4·6,

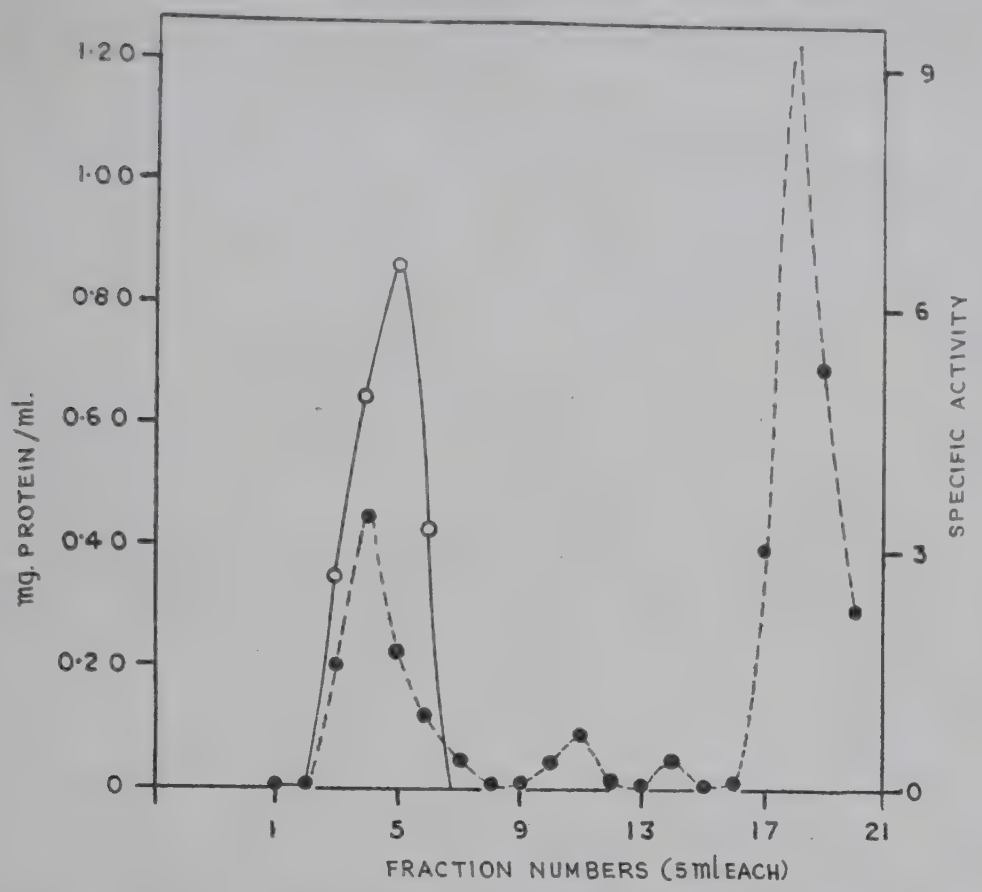

FIG. 1—ELUTION PATTERN OBTAINED BY COLUMN CHROMATOGRAPHY ON DEAE CELLULOSE (●—●, mg. protein/ml.; o—o, specific activity of monoamine-oxidase)

while the initial homogenate had a specific activity of 0·04. The experiments described below were performed with this enzyme preparation. The different steps in the purification procedure are shown in Table 1. A comparison of the activities of the supernate of sonicated mitochondria and the pooled DEAE eluates indicates that among the five monoamines tested (Table 2) serotonin and tyramine are most actively metabolized by rat brain mitochondrial MAO.

Purified rat brain MAO was optimal at *p*H 6·5 while the purified liver enzyme showed an optimum *p*H of 7·0 (Fig. 2). The time-activity curve of brain MAO was found to be linear up to 90 min. (Fig. 3). It is known that liver MAO is inhibited by high substrate concentrations[6], but purified as well as crude brain MAO was found not to be inhibited even by very high substrate concentrations. The effects of different concentrations of various monoamines on both the purified rat brain and liver MAO are shown in Table 3. The hypothesis of eutopic and dystopic complex formation[7] to explain the nature of MAO inhibition is inadequate to explain the different behaviour of MAO of brain and liver with respect to high substrate concentrations unless the occurrence of some structural difference in both the enzymes is assumed.

TABLE 1—STEPS IN THE PURIFICATION OF MAO

[Enzyme activity was assayed in 0·05 M. phosphate buffer *p*H 7·0 in the presence of 0·01 M. tyramine hydrochloride neutralized before use. An initial incubation period of 10 min. at 38° C. followed by the addition of substrate and the [illegible] stopped after 30 min. Ammonia released directly by enzymes or [illegible] by the manometric technique of Braganca *et al.*[14] and all values corrected for blanks. Protein concentration was determined spectrophotometrically at 280 mμ (Warburg and Christian[13]) (using bovine plasma albumin as standard).]

Preparations	Sp. activity μg. NH_3 /mg. protein	Purification (fold)
Homogenate	0·94	1·0
Intact mitochondria	0·39	9·8
Supernate of sonicated mitochondria	1·54	38·5
Combined DEAE-cellulose eluates	4·60	115·0

TABLE 2—COMPARATIVE ACTIVITIES OF RAT BRAIN MAO PREPARATIONS UPON VARIOUS MONOAMINES

[The reaction mixture contained 0·0125 M. semicarbazide neutralized in 0·05 M. phosphate buffer *p*H 7·0. After 10 min. equilibration substrates were added followed by a further period of incubation for 30 min. at 38° C. and aldehyde formed was determined according to the method of Green and Haughton[12]. All values are corrected for blanks. Both the optical density readings (E_{450}) and the calculated aldehyde values employing benzaldehyde as the colorimetric standard are given (for further details *see* Ref. 5)].

Substrate (0·01 m.)	MAO activity of supernate of sonicated mitochondria		MAO activity of pooled eluates after DEAE chromatography	
	E_{450}	μg. Aldehyde formed	E_{450}	μg. Aldehyde formed
Serotonin	0·220	7·5 (100)	0·146	5·00 (100)
Tyramine hydrochloride	0·200	7·0 (93)	0·138	4·75 (95)
Tryptamine hydrochloride	0·174	6·0 (80)	0·110	3·75 (75)
Adrenaline	0·120	4·2 (56)	0·087	3·00 (60)
Benzylamine	0·100	3·5 (47)	0·080	2·75 (55)

Figures in parentheses indicate relative activity with respect to serotonin as 100 and calculated on the basis of aldehyde formed

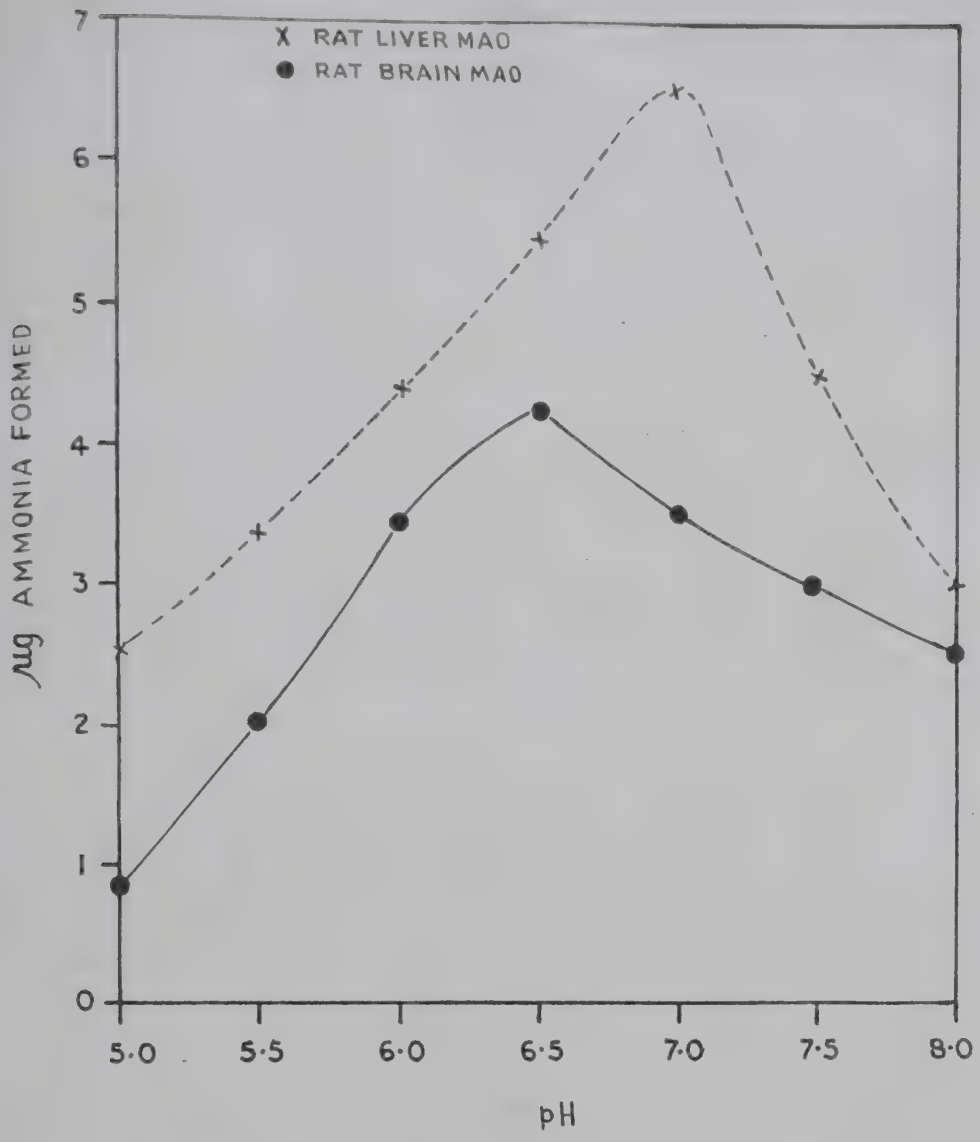

FIG. 2—*p*H ACTIVITY CURVES OF PURIFIED BRAIN AND LIVER MAO

Purified brain MAO was strongly inhibited by pCMB, *o*-phenanthroline and HgCl , while 8-hydroxyquinoline, α-α-dipyridyl, and iodoacetate produced no inhibition (Table 4). Similar inhibition in the case of purified beef liver MAO has been observed[8]. The nature and kinetics of such inhibition on purified brain MAO remains to be further elucidated.

Rat brain MAO was strongly inhibited by eutonyl, tranylcypromine, iproniazid, catron and phenelzine. Table 5 shows the L_{50} values of these compounds with various monoamines as substrate. It is evident from this that change of substrate failed to influence the degree of inhibition by these inhibitors. The kinetics of these inhibitions are under study.

With purified rat liver MAO the nature of tranylcypromine inhibition was found to be competitive and irreversible and depended on a prior incubation of the enzyme with the inhibitor[9]. The inhibitor was found to have a I_{50} value of 2×10^{-7} M. (Table 6).

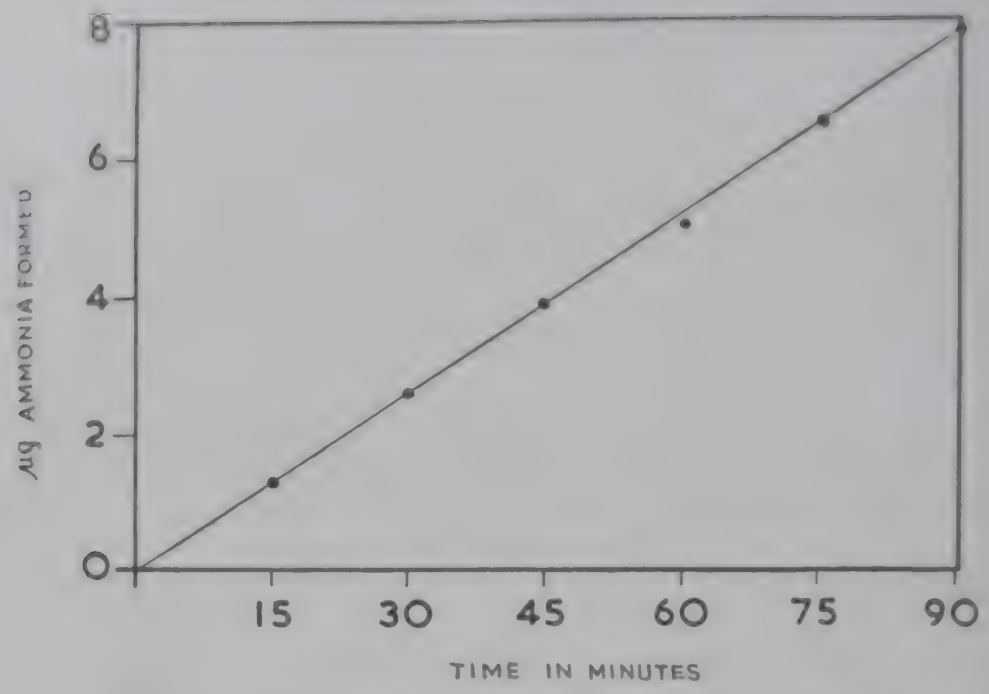

FIG. 3 — TIME-ACTIVITY RELATIONSHIP OF PURIFIED BRAIN MAO

TABLE 3 — EFFECT OF VARIED SUBSTRATE CONCENTRATIONS ON THE ACTIVITY OF PURIFIED BRAIN AND LIVER MAO

All incubations were performed in 0.05 M. phosphate buffer pH 8.5 and other details are given in Tables 1 & 2)

SUBSTRATE CONCENTRATION	ACTIVITY OF BRAIN MAO WITH			ACTIVITY OF LIVER MAO WITH			
	Tyramine*	Tryptamine†	Serotonin†	Benzylamine†	Adrenaline†	Tyramine†	Serotonin†
0·01 M.	3·5	0·092	0·170	0·130	0·143	0·205	0·232
0·02 M.	4·2	0·102	0·190	0·110	0·150	0·182	0·155
0·03 M.	4·7	0·114	0·220	0·090	0·120	0·165	0·125
0·04 M.	5·8	0·135	0·260	0·040	0·090	0·155	0·115

*Ammonia values are given in μg.
†E_{430} values are given

From the above results of inhibition of MAO it appears that eutonyl and tranylcypromine are quite exceptional inhibitors in the field of enzyme mechanics, since these are highly potent at an extremely low concentration.

The present experiments with inhibitors as well as activity of monoamineoxidase towards various monoamines failed to support the suggestion forwarded by some workers regarding the possible existence of multiple forms of monoamineoxidase[10,11].

TABLE 4—EFFECT OF CERTAIN INHIBITORS ON PURIFIED BRAIN MAO

[The enzyme in 0·05 M. phosphate buffer *p*H 6·5 was first incubated with the inhibitors for 15 min. at 38°C. followed by the addition of tyramine (0·01 M). Other details are same as in Table 2]

Inhibitors	Final concentrations	% Inhibition
pCMB	1×10^{-4} M.	85
	5×10^{-5} M.	64
	1×10^{-5} M.	50
o-Phenanthroline	5×10^{-3} M.	60
	1×10^{-3} M.	40
HgCl	5×10^{-5} M.	77
	1×10^{-5} M.	62
α-α-Dipyridyl	5×10^{-3} M.	nil
8-Hydroxyquinoline	5×10^{-3} M.	nil
Iodoacetate	1×10^{-3} M.	nil

TABLE 5—INHIBITION OF PURIFIED BRAIN MAO

(Experimental details are same as in Table 4)

Substrates (0·01 M.)	I_{50} values of inhibitors				
	Eutonyl	*trans*-PCP (Tranyl-cypromine)	Iproniazid	Catron	Phenelzine
Tyramine	5×10^{-8} M.	5×10^{-7} M.	2×10^{-4} M.	1×10^{-4} M.	5×10^{-4} M.
Serotonin	5×10^{-8} M.	5×10^{-7} M.	2×10^{-4} M.	1×10^{-4} M.	5×10^{-4} M.
Tryptamine	5×10^{-8} M.	5×10^{-7} M.	2×10^{-4} M.	1×10^{-4} M.	5×10^{-4} M.

TABLE 6—INHIBITION OF PURIFIED RAT LIVER MAO BY TRANSCYCLOPROPYLAMINE (*trans*-PCP)

(The systems containing enzyme in 0·05M. phosphate buffer *p*H 7·0 with or without *trans*-PCP were first incubated for 15 min. at 38°C. followed by the addition of 0·01M. tyramine and incubated for a further period of 30 min.)

Additions	Inhibition, %
trans-PCP (1×10^{-6} M.)	88
trans-PCP (5×10^{-7} M.)	74
trans-PCP (2×10^{-7} M.)	55
trans-PCP (1×10^{-7} M.)	29
trans-PCP (5×10^{-8} M.)	17

REFERENCES

1. Pugh, C. E. M. & Quastel, J. H., *Biochem. J.*, **31** (1937), 2306.
2. Weiner, N., *Arch. Biochem. Biophys.*, **91** (1960), 182.
3. Blaschko, H., *Pharmac. Rev.*, **4** (1952), 415.
4. Pletscher, A., Proc. Fourth International Congress of Biochemists, Vol. III, (19[illegible] Pergamon Press, London.
5. Guha, S. R. & Krishna Murti, C. R., *Biochem. Biophys. Res. Commun.*, **18** (1965), 350.
6. Cotzias, G. C. & Dole, V. P., *J. biol. Chem.*, **190** (1951), 665.
7. Zeller, E. A., *Ann. N.Y. Acad. Sci.*, **107** (1963), 811.
8. Barbato, G. M. & Abood, L. G., *Biochem. Biophys. Acta.*, **67** (1964), 531.
9. Guha, S. R., *Biochem. Pharmac.* (in press).
10. Gorkin, V. Z., *Nature*, **200** (1963), 77.
11. Ghodera, A., Gorkin, V. Z. & Gridnieva, L. I., *Acta. Biol. Med. Germanica*, **13** (19[illegible] 101.
12. Green, A. L. & Haughton, T. M., *Biochem. J.*, **78** (1961), 173.
13. Warburg, O. & Christian, W., *Biochem. Z.*, **310** (1942), 382.
14. Braganca, B. M., Quastel, J. H. & Schucher, R., *Arch. Biochem. Biophys.*, **52** (1954) 18.

Amine Alkylation, CNS Stimulation and Biochemical Lesion in Schizophrenia

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Because of lack of homogeneity of the schizophrenias, it is extremely doubtful that a single metabolic defect will be found as the causative factor. An advantageous theory would allow a series of related biochemical findings to be correlated into a common behavioral effect with a variety of final biochemical end-products. In developing this thesis we shall attempt to establish three generalizations: (1) that prolonged stimulation whether it be physical, biochemical, or drug induced stimulation will produce hallucinatory states, (2) the chronic schizophrenic is in a state of over-stimulation, and (3) that alkylation of the known biogenic amines which occur in the body may result in compounds with greater CNS stimulant activity and greater lipoid solubility. The prolonged stimulation produced by these substituted biogenic amines results in psychosis.

Thus, the findings of Friedhoff & VanWinkle[1] who report a mescaline-like compound in the urine of schizophrenics can be correlated theoretically with the findings of Brune and Himwich[2] and others who report bufotenine to be a urinary metabolite in schizophrenics. In addition, other abnormal metabolites such as *N*-methylmetanephrine[3] can be postulated as stimulants and potential hallucinogens.

Evidence for Theory of Overstimulation

Goldstein *et al.*[4,5] have shown by a statistical analysis of integrated EEG recordings that the brain waves of the chronic male schizophrenic are relatively non-variant or hyper-regulated. This and other abnormalities are characteristic of continued alerting or stimulation and distinguishes the schizophrenic from the normal subject who has a hyper-regulated

EEG only under LSD-25 or other profound stimulants. We have further shown[6] that treatment of schizophrenic patients with chlorpromazine or perphenazine is accompanied by a return of the brain waves to the normal range of variation and a decrease in their degree of schizophrenic behaviour.

Goldstein *et al.*[4] have also shown that doses of 1 μg./kg. of LSD-25 given to normal volunteers results in a hyper-regulation of the brain waves which then resemble the brain waves of the schizophrenic. This is due to the fact that LSD-25 is a profound and probably specific stimulant of the central nervous system. In rabbits, all of the hallucinogens have been shown to be CNS stimulants[7].

The use of amineoxidase inhibitors as antidepressants has given rise to a new side action as a result of the CNS stimulation which accompanies overdosage[8]. Thus a schizophrenic psychosis may develop in a patient treated with an amineoxidase inhibitor who previously had frequent episodes of depression but no history of schizophrenia. Similarly a recent study of this stimulant in schizophrenics under double-blind test conditions has shown that this medication made the schizophrenic significantly worse[9]. A study of an amineoxidase inhibitor in these laboratories showed that the quantitative brain waves became more hyper-regulated and the behaviour of the schizophrenic patients worsened. The use of iproniazid as a stimulant was in part the direct outgrowth of euphoria and stimulation seen in tuberculosis patients treated with this amineoxidase inhibitor which in overdosage produced a psychotic state.

In this laboratory, Goldstein *et al.*[7] have shown by both behavioral and objective EEG data that schizophrenics can tolerate a cerebral depressant such as an oral dose of 200 mg. of pentobarbital better than the normal volunteer can. In contrast, as judged by emotional outbursts, the schizophrenic tolerated 1 μg./kg. of LSD-25 not as well as the normal volunteer.

The schizophrenic patient has long been known to be an insomniac[10]. This insomnia may precede the psychosis, extend through catatonic states, and be evident in the hospital wards at night if the patients are not treated with tranquillizers. Preliminary brilliance or pre-psychotic stimulation may be one of the first prodromata of a psychotic break[11, 12]. The astute observation of one of our outstanding medical philosophers, Alan Gregg, emphasizes the occurrence of insomnia as the first sign of a psychotic break. "The friends will say that he overworked and had a nervous breakdown whereas the first sign of the psychotic break was the ability and the drive to overwork." The psychosis of prolonged wakefulness may also provide a similar example of the psychotogenic effect of forced overstimulation which is physically induced. While no quantitative data are available, the EEG of prolonged wakefulness is, by inspection, similar to that of the schizophrenic. Thus, these various observations would indicate that the schizophrenic is in a chronic overstimulated state.

Evidence for Enzyme Abnormalities

From the presently available theories[13] the possibility now evolves that the enzymic defect in the various disorders labeled schizophrenia could be characterized by an increase in activity of the alkylating and/or oxidative processes of the body. Since amines are also esterified in the body, as an independent variable, any decrease in acetylating enzymes or other mechanisms which provide esterifying types of detoxication might also result in greater alkylating activity because of the increase in natural substrate of biogenic amines. Since the metabolizing enzymes appear after birth, environmental stress as an occasional cause of psychosis would also be possible. Patients with anxiety can conjugate more glycine with benzoic acid as shown by the hippuric acid excretion test[14,15]. Continued anxiety might exhaust this conjugation mechanism and allow for more alkylation. The only necessary common denominator is that the oxidized or alkylated product of any of the body's natural amines should be an active CNS stimulant which in overdosage would produce hallucinations.

The natural amines which are known at present to provide prototype hallucinogens are all of the phenethyl- or phenindoleamine type. The amines which might provide active hallucinogens are in their probable order of importance: spermidine, agmatine, spermine, histamine, guanidine, indican, skatol, indole, putrescine, pyrrolidine, cadaverine, piperidine and basic polypeptides. Obviously, presently unknown amines may also be involved.

The Methylation Theory

Perhaps the naturally available xanthines provide the oldest example of the stimulant methylated amines wherein the dimethylxanthines, theobromine and theophylline are not particularly stimulant but trimethylxanthine, caffeine, is ingested everywhere for its stimulant action. The CNS anxiety-producing effect of epinephrine compared to norepinephrine should also be cited as an old and familiar example.

An experimental drug, α-methyltryptamine (IT 290, Sandoz), was introduced into clinical trial because of its stimulant effect in animals. We found the stimulant dose in normal volunteers to be 10 mg. orally, but the hallucinogenic dose was 15 to 20 mg.[16]. Thus, with this compound a very narrow range exists between the stimulant and hallucinogenic dose. The α-methyl was much more active than the α-ethyl since 150 mg. of the latter did not produce hallucinations. Other compounds such as MER-16, the methylated analogue of a congener of the stimulant pipradol, is a very active and prolonged hallucinogen[17,18]. Methamphetamine is both more stimulant and hallucinogenic than amphetamine. Since most of the marketed amine stimulants are not methylated, one can assume that perhaps some of the methylated congeners were found by clinical trial to be hallucinogenic.

Among compounds which are not extensively used to date are [illegible] other examples. Methylguanidine is sufficiently stimulant to have been used topically for neuromyography. Codeine (methylmorphine) is more stimulant than morphine and thebaine (dimethylmorphine) is strychnine-like in its CNS effect. The anti-cancer drug dimethylbenzamide has as one of its side actions in man the production hallucinations which from inspection of the EEG may be a result of overstimulation[19]. From this and the morphine examples, the methylation hypothesis may be a valid generalization which includes both *o*- and *N*-methylation. Obviously, the methylation hypothesis applies only to the formation of secondary or tertiary amines and not to quaternary amines which are more water-soluble and excretable. The formation of the natural base, choline, would be a further exception because of its biochemical utility.

Other mechanisms must prevail with the LSD-25 molecule since Abramson[20] reports that DAM-57, *d*-lysergic acid dimethylamide is approximately 1/9 as potent a hallucinogen as LSD-25 in man, and MLD-41 which is 1-methyl LSD-25 is 1/3 as potent. These data agree with the reported rabbit stimulatory potency of these two congeners. In addition, the complete methylation of mescaline to *N, N*-dimethylmescaline results in a compound devoid of hallucinogenic activity (single experiment of Luduena cited by Smythies[13]). This needs further study.

Therapeutic Use of Obligate Methyl Acceptors

Certain therapies which are advocated as primary or adjuvant therapies for schizophrenia involve drugs which act in the body as obligate methyl acceptors and thus may reduce the availability of methyl groups for the formation of stimulant amines. Examples are: nicotinic acid[21–23] which is metabolized by *N*-methylation to trigonelline; piperidine[24] which is *N*-methylated and, deanol which is methylated to choline[25–27]. Finally, a large portion of the common man's tranquillizer, nicotine, acts as a methyl acceptor and is methylated before excretion.

Behavioral Changes with Amino Acids

Two amino acids are known to produce behavioral changes in man. Tryptophane and methionine, or a combination of the two, are most outstanding in this respect[28–30] particularly when given with an amine oxidase inhibitor. From our viewpoint, the negative findings with histidine, phenylalanine, and tyrosine are disappointing but the lack of behavioral changes with these three may be owing to the dosage or to the small sample of schizophrenic patients used.

Balance between Esterification and Alkylation in Disposal of Amines

A well documented defect in the schizophrenic is the inability of some to conjugate glycine with benzoic acid to form hippuric acid[31–36]. Unfortu-

nately this is considered a 'liver function test', but when a battery of such tests are run 'liver function' is found to be essentially normal. Considered alone and not as a test of liver function the hippuric acid test indicates an impaired ability to esterify amines. A similar defect which apparently occurs in some schizophrenics is an increase in non-specific or pseudo-cholinesterase[33]. Such a defect would result in the rapid hydrolysis of biogenic amines which had been detoxified by acetylation. If the acetylating defect extends to choline acetylase then the schizophrenic would have a decrease in the family of parasympathetic neurohumors which are called acetylcholine-like substances.

Primary attention should be given to those cerebral amines which can be metabolized by several alternate routes such as methylation, oxidation or acetylation. Histamine is one such amine which may be methylated on the ring or chain nitrogen or may be acetylated on the chain nitrogen. Thus *N*-acetyl histamine is inactive while, *N*, *N*-dimethyl histamine is more stimulant than histamine[31]. In general, acetylation produces a change toward a sedative effect, increased water solubility and excretion.

This theory may be tested in several ways. The combination which might increase psychotic symptoms are: the methyl donors, methionine, betaine, or choline combined with tryptophane, phenylalanine or histidine. The combination which might decrease a psychotic state are the methyl acceptors such as nicotinic acid, deanol, piperidine and homocysteine.

The objective clinical tests which might be used in schizophrenic patients are: (1) The comparative ability of the schizophrenic to acetylate a standard intravenous dose of sodium sulphadiazine. One gram could be given and the free and combined sulphadiazine determined in the hourly urines for 3 hr. (2) The comparative ability of the schizophrenic to methylate ethanolamine, theobromine or norepinephrine. (A detailed procedure must be evolved in each instance). (3) A repeat of the comparative intravenous hippuric acid test not as a liver function test but as a test of the hippuric acid conjugation process.

Vitamin Deficiencies

Vitamin deficiencies which *per se* result in a decreased esterification of amines should produce mental aberrations. The specific deficiencies could be nicotinic acid (pellagrin psychosis), pantothenic acid (neuroses), vitamin B_{12} (disorientation), etc. Other vitamin factors which might increase the methylation action could increase the tendency to psychosis.

Antipsychotic Drugs Inhibit *N*-Methylation

Chlorpromazine (CPZ) as a prototype of an active antipsychotic drug has been shown by Brown *et al.*[35] to inhibit transmethylation of histamine *in vitro*. Salvador and Burton[36] have found that mice given CPZ show inhibition, *in vitro*, of the enzyme needed to methylate nicotinamide. Heyman[37]

find in schizophrenics that N-methylation of nicotinamide is not increased but certain oxidation products are significantly increased.

Spermidine Congeners

We have recently considered spermidine as an amine which might be alkylated and or partially oxidized to produce an aliphatic CNS stimulant. This might be analogous to the pressor amine *Tuamine* which is 2, amino heptane. Many facets of biological knowledge would favour such a hypothesis.

(1) A spermidine derivative would provide an excellent geometric fit to the three types of antipsychotic drug molecules which are effective in schizophrenia. These are the chemically dissimilar reserpine, butyrophenones and phenothiazines.

(2) Spermidine is elaborated by the pancreas, prostate, and lactating mammary glands. The spermidine released by the prostate at puberty might initiate the onset of schizophrenia in some young adults. The schizophrenic postpartum reaction might correlate with lactation in the susceptible female.

(3) The methylated spermidines synthesized in our laboratory have been found to be stimulant when tested in the rabbit.

(4) Kremzner & Pfeiffer[35] have shown that the interfering amine in the determination of brain histamine by the fluorescence method is spermidine. Thus, *o*-phthaldehyde can now be combined with column chromatography to analyse for both amines in brain tissue. Kremzner[35] have determined the rat whole brain level of spermidine to be 40 μg./g., a level approximately 1000-fold greater than that of histamine and 100-fold greater than the serotonin levels reported for the whole brain of the rat[36]. The various agents which change brain spermidine can now be determined.

In a recent report from Moruzzi's laboratory[41] spermine injections have been found to increase RNA and DNA in the developing chick embryo and polyamines such as spermine and spermidine may act therefore as a stabilizing agent for the synthesis of nucleic acids. Since the neuronal RNA is now thought to be important for memory, any altered spermidine metabolism might be important in the schizophrenias.

Alkylation other than Methylation

We have already indicated that in alpha alkylated tryptamines the ethyl congener was less active than the methyl. Furthermore, nitrogen or oxygen ethylation as a normal metabolic process has not been described. The alkylation of primary amines with body aldehydes is however a definite possibility which has received little or no attention. We refer to the formation of Schiff's bases.

$$R-NH_2+HC(=O)-CH_3 = R-N=C(H)-CH_3+H_2O$$

This type of compound would have a greater affinity for the receptors and would also have free passage of the blood-brain barrier because of greater lipid solubility. The reversibility of this reaction migh allow a chemical reaction at receptors so that a more prolonged pharmacological effect would be obtained. Some observations would favour this reaction in that Heath *et al.*[11] have reported that the schizophrenic behavioral state worsens with disulphuram (antabuse) therapy which is known to increase blood acetaldehyde.

SUMMARY

The chronic male schizophrenic has been shown to be consistently more aroused or stimulated than the non-psychotic controls. This behavioral state might be produced by an alkylated biogenic amine since all hallucinogens are CNS stimulants and methylation is shown to increase the CNS stimulant effect of most drug molecules. The level of spermidine in animal brains is 100-fold greater than that of serotonin. Spermidine may be the common denominator which could be blocked from the CNS by the reserpine, phenothiazine and butyrophenone types of antipsychotic compounds. Alkylated spermidines are stimulant. Alternate to methylation, the combination of primary amines with acetaldehyde must be investigated as a primary amine transport and receptor-affinity increasing reaction.

REFERENCES

1. Friedhoff, A. J. & VanWinkle, E., *Nature*, **194** (1962), 897.
2. Brune, G. G. & Himwich, H. E., *Archs. gen. Psychiat.*, **6** (1962), 82.
3. Perry, T. L., *Science*, **139** (1963), 587.
4. Goldstein, L., Murphree, H. B., Sugerman, A. A., Pfeiffer, C. C. & Jenney, E. H., *Clin. Pharmac. Ther.*, **4** (1963), 10.
5. Goldstein, L. & Beck, R. A., *Int. Rev. Neurobiol.*, **8** (1965).
6. Goldstein, L., Sugerman, A. A., Stolberg, H., Murphree, H. B. & Pfeiffer, C. C., *EEG & Clin. Neurophys*, **19** (1965), 350.
7. Pfeiffer, C. C., Goldstein, L., Murphree, H. B. & Sugerman, A. A., *Am. J. Psychiat.*, **121** (1965), 1147.
8. Douglas Goldman, personal communication.
9. Unpublished data (this laboratory).
10. Rodman, F., Griffith, R. & Sparks, C., *Confinia. Psychat.*, **5** (1962), 189.
11. Parfit, D. N., *J. mental Sci.*, **102** (1956), 708.
12. Goldberg, E. M. & Morrison, S. L., *Br. J. Psychiat.*, **109** (1963), 463.
13. Smythies, J. R., *Post-grad. med. J.*, **39** (1963), 26.
14. Persky, H., Grinker, R. R. & Mirsky, I. A., *J. clin. Invest.*, **29** (1950), 110.
15. Johannsen, W. J., Friedman, S. H., Feldman, E. I. & Negrete, A., *Psychosom. Med.*, (1962).
16. Murphree, H. B., Dippy, R. H. & Pfeiffer, C. C., *Clin. Pharmac. Ther.*, **2** (1961), 722.
17. Pfeiffer, C. C., Murphree, H. B., Jenney, E. H., Robertson, M. G., Randall, A. H. & Bryan, L., *Federation Proceedings*, **17** (1958), 403.
18. Pfeiffer, C. C., Murphree, H. B., Jenney, E. H., Robertson, M. G., Randall, A. H. & Bryan, L., *Neurology*, **9** (1958), 249.

19. Weiss, A. J., Mangall, E. L., Koltes, J. A., White, J. C. & Jackmis, I. G., **136** (1961), 151.
20. Abramson, H. A., *J. Psychol.*, **48** (1959), 65.
21. Hoffer, A., Niacin Therapy in Psychiatry, C. C. Thomas, Springfield, (1962).
22. Hoffer, A. & Osmond, H., *Acta Psychiat. Scand.*, **40** (1964), 171.
23. Ivanova, R. A., Milstein, G. T., Smirnova, L. B. & Fantchenko, N. D., *J. N... & Psychiat.* of C. C. Korsakoff, **64** (1964), 3.
24. Tasher, D. C., Abood, L. G., Gibbs, P. A. & Gibbs, E., *J. Neuropsych.*, **1** (1960), 264.
25. Pfeiffer, C. C. *et al.*, *Science*, **126** (1957), 610.
26. Murphree, H. B., Jenney, E. H. & Pfeiffer, C. C., *Proc. ARNMD*, **37** (1959), 204.
27. Pfeiffer, C. C., *Psychosomatics*, **2** (1961).
28. Park, L. C., Baldessarini, R. J. & Kety, S. S., *Arch. gem. Psychiat.*, **12** (1965), 346.
29. Alexander, F., Curtis, G. C., Sprince, H. & Crosley, A. P., *J. Nerv. Ment.* ..., **137** (1963), 135.
30. Brune, G. G. & Himwich, H. E., *J. Nerv. Ment. Dis.*, **134** (1963), 447.
31. Quastal, J. H. & Wales, W. T., *Lancet.*, **2** (1933), 301.
32. Davies, D. R. & Hughes, T. P. E., *Lancet.*, **1** (1940), 430.
33. Gal, E. M., *Nature*, **198** (1963), 1118.
34. Goldstein, L., Pfeiffer, C. C. & Munoz, C., *Fed. Proc.*, **23** (1964), 1113.
35. Brown, D., Tomchick, R. & Axelrod, J. V., *J. biol. Chem.*, **234** (1959), 2048.
36. Salvador, R. A. & Burton, R. M., *Biochem. Pharmac.*, **14** (1965), 1185.
37. Heyman, J. J., *Tr. N. Y. Acad. Sci.*, **26** (1964), 354.
38. Kremzner, L. T. & Pfeiffer, C. C., Biochemical Pharmacology (in press).
39. Kremzner, L. T., (unpublished data).
40. Garrattini, S. & Valzelli, L., Serotonin, Elsevier Press, (1965), 377.
41. Heath, R. G., Nesselhof, W., Bishop, M. P. & Byers, L. W., *Dis. Nerv. Syst.*, **26** (1965), 99.

Studies in 4-Substituted 2,3-Polymethylenequinolines

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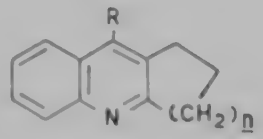
The work described in this paper is based on the biological activity of 5-aminotetrahydroacridine (THA). This compound has a wide spectrum of biological activities, the most important of which are anticholinesterase[1], morphine-antagonism[2], respiratory stimulant[2c], analgesic and hallucinogen counteracting actions[3]. 5-Aminoacridine has a strong antibacterial action but a rather weak pharmacological action, while THA compound has weak bacteriostatic action[4] but possesses the wide spectrum of biological activities mentioned above. The aplanarity of one of the rings thus seemed responsible in some way for the enhanced pharmacological activity of this molecule.

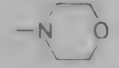
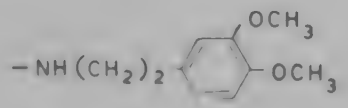
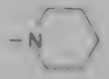
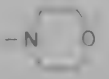
The pharmacological profile of THA indicated that its various pharmacological actions need not necessarily be associated together, and it seemed possible to dissociate the various activities of the molecule by structural alteration. These considerations thus made this compound an interesting model for further exploration.

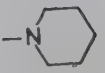
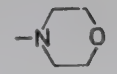
But for an old report of the analeptic action of 3,4-dihydro-1, 2-benzacridine-5-carboxylic acid (tetrophan)[5] and local anaesthetic activity for the diethylamide of 1,2,3,4-tetrahydroacridine-5-carboxylic acid[6] and a report published during the course of this work on the analeptic activity of amino-cycloheptaquinoline[7], not much is known about the pharmacology of these compounds. Quite recently Brian and Souther[8] have reported the synthesis of a few substituted 5-amino-1,2,3,4-tetrahydroacridines but no data concerning their biological activity are reported.

Systematic variations were introduced in various parts of the molecule of THA. First, the 5-amino group was substituted to vary the charge density around this part of the molecule. In addition, the corresponding amides were prepared. Next, the size of the saturated ring was varied to delineate the role of aplanarity on biological activity—as a change to five membered ring would make the molecule more or less planar, while increase to seven or

TABLE 1



S. No.	n=	R=	Approx. LD_{50} (mice) *mg./kg. i.p.*	Prominent effects	% Effect on pentobarbitone (60 mg./kg. i.p. hypnosis of one LD_{50} dose)	Analgesic activity (rats)		Local anaesthetic activity (2% soln. rabbit's cornea)
						Dose *mg./kg. i.p.*	% Intensity/duration *min.*	Intensity/duration
1	1		45	Sedation, episthotonus, clonic convulsions, antipyresis	0	4.5	0	0
2	2	NHC_4H_9	50	Polypnea, hyper-reflexia, clonic convulsions, antagonized reserpine induced ptosis	0	5	50/30	Complete/180
3	2		100	Hyper-reflexia, cyanosis, b.p. ↑ 90 mm. (T)	0	10	0	Complete 180
4	2		50	Polypnea, clonic convulsions	50	5	0/0	Complete/180
5	2		100	Polypnea, clonic convulsions, increase in rate and depth of respiration for 30 min.	0	10	80/60	0

6	2	$-CON(C_2H_5)_2$	250	Polypnea, mixed convulsions, hyper-reflexia	0	25	60/20	Partial/15
7	3		70	Clonic & tonic convulsions, hyper-reflexia, antagonized reserpine induced ptosis and hypothermia	+50	7	0	Complete/100
8	3		15	Hyper-reflexia, clonic and tonic convulsions	−80	1.5	0	Partial/40
9	3		50	Polypnea, clonic & tonic convulsions, marked increase in rate & depth of respiration exceeding 60 min. in anaesthetized cats, partially antagonized reserpine-induced ptosis, reserpine potentiated its convulsive action	−90	5	70/30	0
10	3	$-CON(C_2H_5)_2$	250	Polypnea, hyper-reflexia, clonic convulsions	+100	25	40/40	0
11	2	$-NH_2$	30	Tail lashing followed by depression, piloerection, tremors, slight increase in rate of respiration in anaesthetized cats, antagonized reserpine-induced ptosis, reserpine potentiated its convulsive action	0	3	60/45	Complete/120

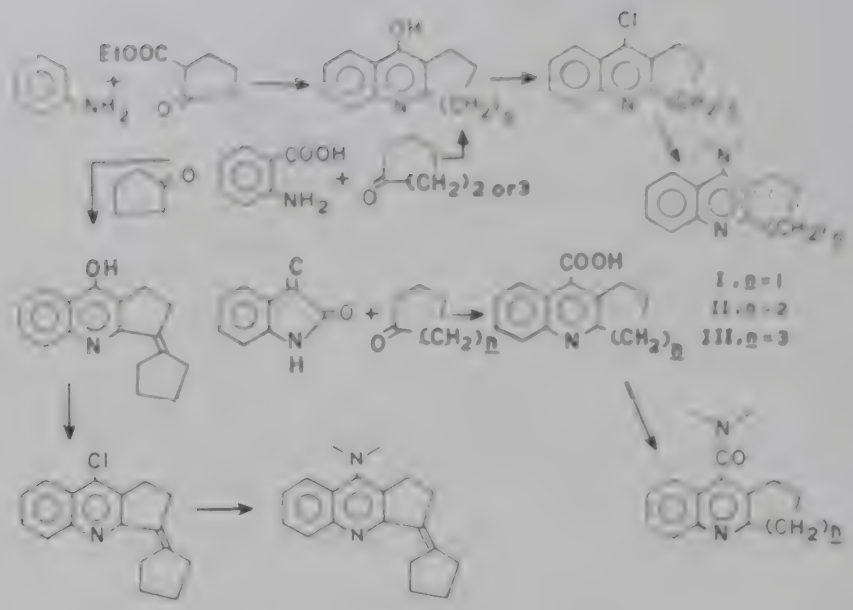


FIG. 1

higher membered rings would increase the aplanarity. And finally, various substituents were introduced in the benzene ring. The method of synthesis of the various compounds is described in Fig. 1.

The results of the primary pharmacological screening of some selected compounds are given in Table 1. In the gross behaviour, most of the compounds showed a central stimulant action as evidenced by hyper-reflexia, hypersensitivity to touch, 'Straub' phenomenon, persistent clonic and, in certain cases, tonic convulsions. The other prominent activities shown by these compounds were the effect on barbiturate narcosis, analgesic action, stimulation of respiration and local anaesthetic activity, without any prominent effect on the B.P. There was considerable dissociation of various activities in different compounds. The trimethylene quinolines (I) were in general devoid of any prominent activity. The analgesic activity was more marked in the tetramethylene quinolines (II) and was quite significant both in the 4-(4-morpholinyl) (5) and 4-(1-piperidyl) (4) compounds. Some of the tetramethylene compounds showed a potentiation of the barbiturate hyponsis. The local anaesthetic activity was present mainly in the piperidyl (4) and pyrrolidyl compounds of the tetramethylene quinolines and was much stronger than that of the corresponding amide including the diethylamide (6) mentioned earlier by Magidson and Travin[6] as a local anaesthetic. The analeptic activity was more prominent in the pentamethylene compounds (III). In the pentamethylene compounds, there was further interesting dissociation of antibarbiturate and respiratory stimulant activities—the piperidyl and morpholinyl compounds (8 & 9) both counteracted barbiturate hypnosis but the latter, in addition, had a marked respiratory stimulant action.

As 4-(4-morpholinyl)-2,3-pentamethylenequinoline (9) showed promising analeptic activity in the primary screening, the pharmacological activity of this compound was studied in greater detail. In mice at one half of LD_{50} it could reduce the sleeping time of 60 mg./kg. of pentobarbitone by 85 per cent (Table 2), and there was cross protection against barbiturate toxicity at different dose levels (Table 3).

At one half of LD_{50} the sleeping time of ethanol (0·4 g./kg., normal sleeping time 40 min.) hypnosis was reduced by 88 per cent. From ethanol, urethane and chloral hydrate hypnosis at one LD_{50} dose the arousal was instantaneous, but no protection against the toxicity of the drug was afforded.

Respiratory Stimulant Action

The respiratory stimulant action of compound (9) was unaffected by carotid and aortic denervation. This compound had no effect on electrical transmission through the superior cervical ganglion of cat. In rabbits it could prevent the death due to respiratory failure by thiopentone, pentobarbitone and phenobarbitone, and the latter two barbiturates in turn prevented the death due to very high doses of the compound. It was more active than THA (Table 4). Nikethamide and 'Micoren' at similar

TABLE 2—EFFECT OF COMPOUND (9) AGAINST HYPNOSIS PRODUCED BY 60 mg./kg. i.p. OF PENTOBARBITONE IN MICE

(Twelve mice used at each dose level. Control sleeping time 80–120 min. at 23°)

Dose of compound 9 in LD_{50}	% Reduction in sleeping time
$\frac{1}{4}$	36
$\frac{1}{2}$	85
1	88

TABLE 3—EFFECT OF COMPOUND (9) ON PENTOBARBITONE TOXICITY IN MICE

(no. dead/no. used)

Dose of compound 9 in LD_{50}	Dose of pentobarbitone, mg./kg. i.p.					
	115	125	135	145	175	200
Control	3/9	9/11	10/10	..	..	..
1	..	..	..	..	0/10	8/12
2	2/12	..	..	2/12	6/12	6/12
3	..	..	4/12	0/10	..	..
4	4/12	6/12	4/12	3/12	..	..
6	..	..	6/6	6/6	6/6	..

TABLE 4—ANTAGONISM OF RESPIRATORY DEPRESSION IN RABBITS

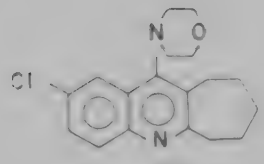
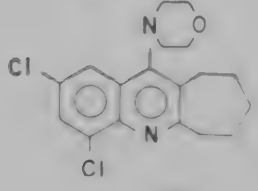
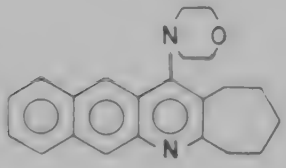
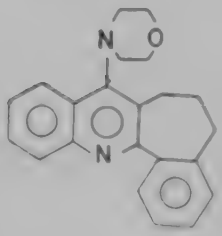
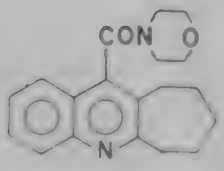
Rabbit No.	Depressant (Route of administration	Dose *mg./kg.*	Rate of respiration after depressant *no./min.*	Stimulant	Dose *mg./kg. i.v.*	Rate of respiration after stimulant *no./min.*	Comments
1	Pentobarbitone (i.p.	75	20	Compound 9	40	40	Survived
2	do	75	12	do	40	60	do
3	do	75	9	THA	40	22	Died
4	do	75	0	do	20	20	Survived
5	do	75	20	do	20	0	Died
6	do	75	20	Metrazole	90	33	Survived
7	do	75	10	do	150	20	do
8	do	75	15	Micoren	65	14	Died
9	do	75	20	do	60	16	do
10	do	75	20	do	120	0	do
11	do	75	20	Nikethamide	100	10	do
12	do	75	14	do	130	14	do
13	do	75	10	do	100	20	do

14	do	75	20	do	—	—	do
15	Thiopentone (i.v.)	30	Stopped	Compound 9	5	80	Survived
16	do	50	do	do	5	50	do
17	do	50	do	do	5	66	do
18	do	35	do	THA	4	0	Death
19	do	25	do	Metrazol	40	0	do
20	do	50	do	do	90	0	do
21	Thiopentone (i.v.)	50	Stopped	Metrazol	60	0	Death
22	do	25	do	do	—	—	do
23	Phenobarbitone (i.v.)	300	do	Compound 9	60	85	Survived
24	do	300	do	do	60	50	do
25	do	300	do	THA	20	0	do
26	do	300	do	do	—	—	Death
27	Morphine (i.v.)	10	28	Compound 9	2.4 (in a divided dose)	60	Survived
28	do	10	24	do	2 (in 3 divided doses)	80	do
29	do	10	20	do	2.6 (in 4 divided doses)	70	do
30	do	10	20	THA	3.0 (in 6 divided doses)	40	do
31	do	10	20	do	3.0 (in 6 divided doses)	50	do

TABLE 5– EFFECT OF COMPOUND (9) ON MORPHINE-INDUCED ANALGESIA IN RATS

Dose of morphine *mg./kg.*	Dose of compound *mg./kg. i.p.*	Analgesia	
		% Intensity	Duration *min.*
4·5 s.c.	..	60	30
4·5 s.c.	2	80	60
	2	0	0
20 i.p.	..	100	>60
20 i.p.	5	100	>60

TABLE 6

S. No.	Compound	Approx. LD_{50} (mice) *mg./kg. i.p.*	Gross effects	% Effect on pentobarbitone (55 *mg./kg. i.p.* hypnosis by one half LD_{50} dose)
12		800	At lower dose no marked effect. At higher dose marked depression, hypothermia 10°F.	−43
13		1500 (Agar suspension	No prominent effects	−45
14		400 (Agar suspension)	No prominent effects	+17
15		800	No prominent effect, at high doses hypothermia 16°F.	+80
16		250	Polypnea, mixed convulsions, catatonia, mydriasis	+50

doses could not prevent the pentobarbitone-induced toxicity, while pentylenetetrazole could check the death, but the increase in respiration was not as marked as with compound (9).

The respiratory stimulant action was also evident in anaesthetized cats and rabbits, where depressed respiration was produced by loading doses of morphine, pentobarbitone and chloralose.

In view of the possibility of using this compound as an antidote for morphine depression, its effect on morphine-induced analgesia in rats was studied and the results given in Table 5 show that this compound did not counteract morphine-induced analgesia; if at all, it increased the analgesia to some extent. It, therefore, may possibly be used to counteract morphine-induced depression in cases where heavy doses or morphine have to be used to produce deep analgesia.

Some of the other structural analogues synthesized in this study and their pharmacological activity are described in Table 6. The results show that introduction of Cl in position 6 & 6,8 (12 & 13) somewhat reduces the activity, without in any way altering the pattern of activity. Increase in the planar area of the molecule (14 & 15), however, completely alters the pattern of activity, the compounds are no longer stimulants and analeptics, and show, on the other hand, depressant action and potentiate barbiturate hypnosis.

REFERENCES

1. (*a*) SHAW, F. H. & BENTLEY, G. A., *Aust. J. exp. Biol. Med. Sci.*, **31** (1953), 573.
   (*b*) DE LA LANDE, I. S. & BENTLEY, G. A., *ibid.*, **33** (1955), 555.
   (*c*) HEILBRONN, E., *Acta. Chem. Scand.*, **15** (1961), 1386.
2. (*a*) SHAW, F. H. & BENTLEY, G. A., *Med. J. Aust.*, **2** (1949), 868.
   (*b*) SHAW, F. H. & BENTLEY, G. A., *Nature*, **169** (1952), 712.
   (*c*) SHAW, F. H. & BENTLEY, G. A., *Aust. J. exp. Biol. Med. Sci.*, **33** (1955), 143.
   (*d*) SHAW, F. H., GERSHON, S. & BENTLEY, G. A., *J. Pharm. Pharmac.*, **9** (1957), 666.
3. GERSHON, S., *Nature*, **186** (1960), 1072.
4. The Acridines by Adrien Albert, Edward Arnold & Co. (1951), 300.
5. (*a*) HESSE, E., *Arch. exp. Path. Pharm.*, **111** (1962), 68.
   (*b*) POHL, J. & HESSE, E., *Wochschr. Klin.* **4** (1926), 344; *Chem. Abstr.*, **20** (1926), 2204.
6. MAGIDSON, O. & TRAVIN, A. I., *J. Gen. Chem.* (U.S.S.R.), **7** (1937), 842.
7. PLOTNIKOFF, N., KEITH, J., HEIMANN, M., KEITH, W. & PERRY, C., *Arch. int. Pharmacodyn.*, **146** (1963), 406.
8. BRIAN, W. P. & SOUTHER, B. L., *J. med. Chem.*, **8** (1965), 143.

N-Aralkylanthranilic Acid Derivatives as CNS Depressants

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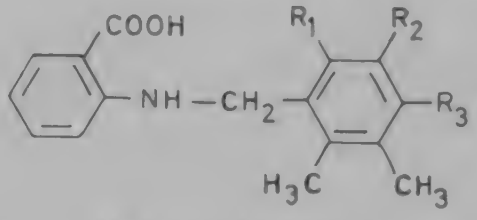
Introduction

N-Aralkylanthranilic acids have come into prominence recently on account of their analgesic, antipyretic and anti-inflammatory activity.

Winder *et al.* (1962) tested a number of substituted anthranilic acids and found *N*-2, 3-xylylanthranilic acid (mefenamic acid) to be the most potent.



Mefenamic acid

It has been reported to possess analgesic, antipyretic and anti-inflammatory activity.

Chemical investigations of mefenamic acid have been carried out by Cass and Frederik (1963) on patients suffering from various types of painful conditions, and by Kendall (1964) on patients suffering from cervical spondylosis. They found mefenamic acid useful in relieving clinical pain. Subsequently it has been introduced into therapeutics both in the USA and the UK for relief of pain.

As part of the research programme at the Regional Research Laboratory, Hyderabad, on the synthesis of compounds having an action on the central nervous system, a number of *N*-aralkylanthranilic acids have been synthesized as intermediates for the synthesis of seven-membered ring systems.

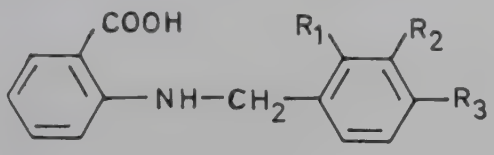
In view of the close structural similarity to mefenamic acid, it was thought worthwhile to screen these *N*-aralkylanthranilic acids for their

action on the central nervous system, with particular reference to their analgesic and antipyretic activity, at the Gandhi Medical College, Hyderabad.

CHEMISTRY

The compounds screened in the present investigation have been classified into three groups based on their structure, viz. (i) *N*-benzylanthranilic acids; (ii) *N*-α-alkylbenzylanthranilic acids, and (iii) *N*-phenethylanthranilic acids (Tables 1, 2 and 3).

TABLE 1—*N*-BENZYLANTHRANILIC ACIDS



Compound	R_1	R_2	R_3	m.p.
RH 1	H	H	H	173-4°
RH 2	Cl	H	H	182-3°
RH 3	H	H	Cl	130-1°
RH 4	Cl	H	Cl	185-6°
RH 5	H	Cl	Cl	153-4°
RH 10	H	H	OCH_3	123-4°
RH 11	H	OCH_3	OCH_3	90-1°
RH 12	H	-O-CH_2	-O-	104-5°

TABLE 2—*N*-α-ALKYLBENZYLANTHRANILIC ACIDS

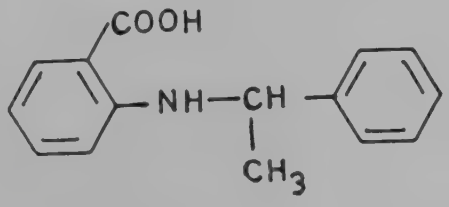
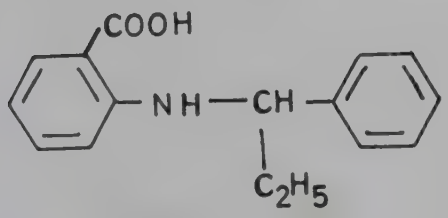
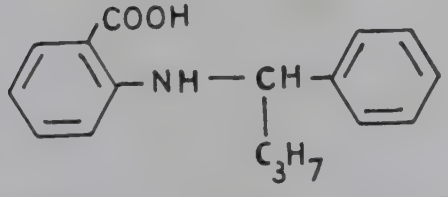
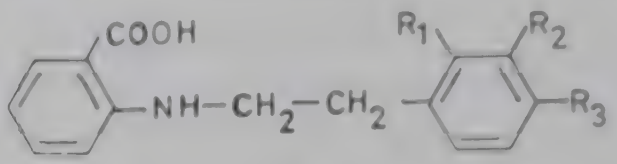
Compound	Structure	m.p.
RH 6		159-60°
RH 7		137-8°
RH 13		107-8°

TABLE 3—*N*-PHENETHYLANTHRANILIC ACIDS



Compound	R_1	R_2	R_3	m.p.
RH 8	H	H	H	116-7°
RH 22	H	H	CH_3	144-6°
RH 14	H	H	OCH_3	122-3°
RH 15	H	OCH_3	OCH_3	108-9°

PHARMACOLOGY

Methods

All the 15 compounds were subjected to primary screening for gross observations. Groups of four mice, two males and two females, each weighing between 20 and 25 g., were injected intraperitoneally with a suspension of the drugs in 4% acacia gum at a dose of 100 mg./kg., and then observed for 2 hr. Most of the compounds produced hypoactivity, and a few of them drowsiness which could be observed within 10 to 15 min. of administration of the drug. With most of the drugs, normal activity was resumed within 30 min., and a few within 60 min. after injection. Analgesic effect was tested by: (i) the tail-clip (mechanical) method; (ii) the hot plate method; (iii) the radiant heat method; (iv) writhing induced by chemical agents, and (v) the electric shock method.

To economize on animals, mice were mainly used for the analgesic tests, and to save time the intraperitoneal route was preferred. On an average, 50–60 mice of both sexes in groups of 10 were used for each drug under study. An arbitrary dose of 100 mg./kg. was selected for each compound as it was about one-sixth of the LD_{50} for one of the drugs, viz. RH 8. As the compounds were all insoluble in water, they were administered in the form of a suspension in 4 per cent acacia gum. The criterion chosen for effective analgesic activity was that an at least 30 per cent positive analgesic response in mice should be observed by the first three methods, viz. tail-clip, hot plate and radiant heat. Three standard analgesics were tested alongside; codeine, though a narcotic analgesic, was included as it exhibits activity by all the three methods; aspirin as it is a widely used and is a clinically effective non-narcotic analgesic, and mefenamic acid as the main standard of comparison as it is very closely related in chemical structure to the compounds under investigation and has been recently introduced into therapeutics as a useful analgesic agent.

Codeine showed 100 per cent positive analgesic response in mice by all the three methods (Table 4). Mefenamic acid appeared to fulfil the criterion laid down; it showed more than 30 per cent positive analgesic response by all the three methods selected. Aspirin did not equal mefenamic acid in the doses chosen for comparison.

Analgesic Activity of *N*-Benzylanthranilic Acids

By the criterion laid down, only RH 1 (*N*-benzylanthranilic acid) appeared to be an effective analgesic from amongst the compounds listed in Table 1. In this group, compound RH 3 (*N*-*p*-chlorobenzylanthranilic acid) also deserves consideration for further investigation as it provided 60 per cent positive analgesic response by two methods, viz. tail-clip and radiant heat (Table 5).

TABLE 4—PERCENTAGE OF POSITIVE ANALGESIC RESPONSES IN MICE BY VARIOUS METHODS AT 30, 60 AND 90 min. AFTER DRUG ADMINISTRATION

Drugs 100 *mg./kg. i.p.*	Tail-clip *min.*			Hot plate *min.*			Radiant heat *min.*		
	30	60	90	30	60	90	30	60	90
Codeine	**100**	100	60	**100**	100	90	**100**	70	60
Aspirin	**20**	10	0	**10**	10	0	**10**	0	0
Mefenamic acid	**36**	28	12	**45**	15	5	**30**	30	30

TABLE 5—PERCENTAGE OF POSITIVE ANALGESIC RESPONSE IN MICE BY VARIOUS METHODS AT 30, 60 AND 90 min. AFTER DRUG ADMINISTRATION

Drug 100 *mg./kg. i.p.*	Tail-clip *min.*			Hot plate *min.*			Radiant heat *min.*		
	30	60	90	30	60	90	30	60	90
RH 1	**31**	8	0	**30**	**30**	30	10	**40**	20
2	**23**	15	8	**40**	10	0	**20**	20	0
3	**60**	40	23	**10**	10	10	**60**	50	40
4	19	**19**	12	10	**20**	20	30	**60**	50
5	36	**45**	9	**20**	20	10	0	**8**	0
10	**21**	14	7	**20**	20	20	**20**	20	10
11	**29**	14	7	0	0	0	0	0	0
12	**43**	14	14	0	0	0	**10**	10	0

Analgesic Activity of N-α-Alkylbenzylanthranilic Acids

According to the criterion adopted for effective analgesic activity, two compounds RH 7 (N-α-ethylbenzylanthranilic acid) and RH 13 (N-α-propylbenzylanthranilic acid) from group 2 appeared to be potent and of these two RH 7 was more powerful (Table 6).

Analgesic Activity of N-Phenethylanthranilic Acids

Amongst the compounds of group 3, listed in Table 7, RH 8 (N-phenethylanthranilic acid) alone appeared to fulfil the criterion laid down for effective analgesic activity; RH 15 [N-(3,4-dimethoxyphenyl) ethylanthranilic acid] also needs some consideration as it showed 50 per cent positive analgesic response by the tail-clip method and 40 per cent by the hot plate method.

Out of a total of 15 compounds tested from all the three groups four compounds appeared to fulfil conditions for effective analgesic activity. They are: RH 1 (N-benzylanthranilic acid), RH 7 (N-α-ethylbenzylanthranilic acid), RH 13 (N-α-propylbenzylanthranilic acid) and RH 8 (N-phenethylanthranilic acid).

TABLE 6—PERCENTAGE OF POSITIVE ANALGESIC RESPONSES IN MICE BY VARIOUS METHODS AT 30, 60 AND 90 min. AFTER DRUG ADMINISTRATION

Drug 100 *mg./kg. i.p.*	Tail-clip *min.*			Hot plate *min.*			Radiant heat *min.*		
	30	60	90	30	60	90	30	60	90
RH 6	**19**	12	6	**10**	10	10	**10**	10	10
7	**63**	37	16	**40**	40	40	**40**	40	33
13	**57**	43	29	**60**	40	40	**29**	21	21

TABLE 7—PERCENTAGE OF POSITIVE ANALGESIC RESPONSES IN MICE BY VARIOUS METHODS AT 30, 60 AND 90 min. AFTER DRUG ADMINISTRATION

Drug 100 *mg./kg. i.p.*	Tail-clip *min.*			Hot plate *min.*			Radiant heat *min.*		
	30	60	90	30	60	90	30	60	90
RH 8	**59**	31	15	**44**	24	15	**53**	53	47
22	**13**	13	0	**13**	13	7	0	0	0
14	**21**	14	7	0	0	0	0	0	0
15	**50**	21	14	**40**	0	0	**10**	10	10

Comparative Analgesic Activity of Selected Compounds

When we tested RH 1, RH 7, RH 13 and RH 8 against one another we found that RH 8 was most active by the hot plate and radiant heat methods, though RH 7 was a little more active than RH 8 by the tail-clip method.

RH 8, when compared with the three standard analgesic agents selected, was seen to be more active than both aspirin and mefenamic acid by the tail-clip and radiant heat methods. By the hot plate method, RH 8 was more active than aspirin. By all the three methods RH 8 appeared to be less active than codeine (Table 8).

Analgesic Activity of Compound RH 8

Dose-response relationship of RH 8 was studied for all the three methods by i.p. administration of 50, 75, 100, 125 and 150 mg./kg. in mice. The average ED_{50} given by these methods was approximately 90 mg./kg. (Fig. 1).

Amongst the drugs under investigation, RH 8 being most active was also tested for analgesic activity by the writhing test in mice. It was observed that RH 8 was more active than mefenamic acid in protecting mice from writhing by both acetic acid and *p*-benzoquinone (Table 9).

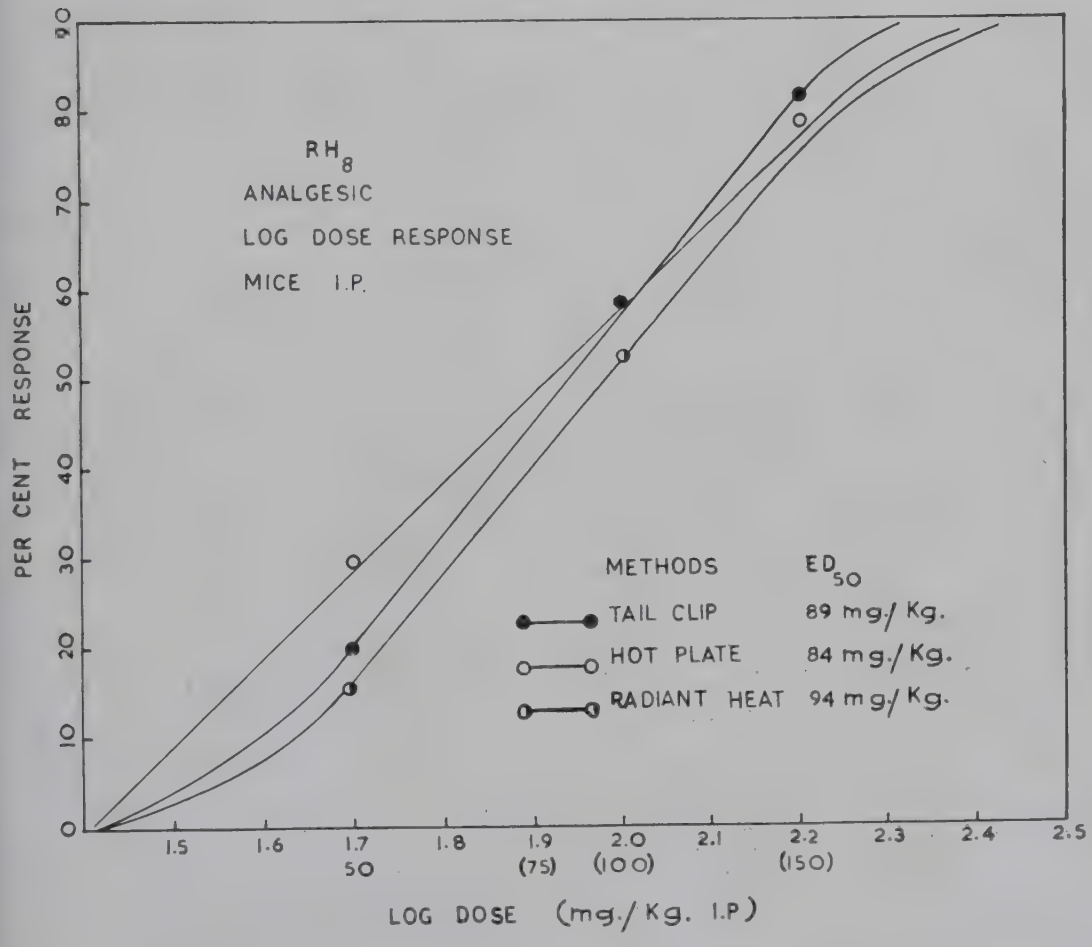


FIG. 1

TABLE 8—PERCENTAGE OF POSITIVE ANALGESIC RESPONSES IN MICE AT 30, 60 AND 90 min.

Drug 100 mg./kg. i.p.	Tail-clip min.			Hot plate min.			Radiant heat min		
	30	60	90	30	60	90	30	60	90
Codeine	**100**	100	100	**100**	100	90	**100**	70	60
Aspirin	**20**	10	0	**10**	10	0	**10**	0	0
Mefenamic acid	**36**	28	12	**45**	15	5	**30**	30	30
RH 8	**89**	31	15	**44**	24	15	**53**	53	47

TABLE 9—PERCENTAGE OF MICE PROTECTED FROM WRITHING AT 10 AND 30 min. AFTER i.p. ADMINISTRATION OF CHEMICAL AGENTS

(Writhing test by chemical agents)

Drugs (100 mg./kg. i.p.) given 30 min. before administration of chemical agent	Chemical agent			
	Acetic acid		*p*-Benzoquinone	
	10 min.	20 min.	10 min.	20 min.
Mefenamic acid	70	60	67	67
RH 8	80	80	80	73

TABLE 10—PERCENTAGE OF POSITIVE ANALGESIC RESPONSES IN MICE AT 30, 60 AND 90 min. AFTER DRUG ADMINISTRATION—ELECTRO SHOCK METHOD

Drugs	i.p. Dose/kg.	30 min.	60 min.	90 min.
Codeine	50 mg.	**40**	40	40
Mefenamic acid	100 mg.	**10**	10	0
RH 8	100 mg.	**20**	10	3

As the electro-shock method is also considered to be one of the most reliable methods for testing analgesic activity, it was decided to test RH 8 by this method. From the results, it is observed that RH 8 in 100 mg./kg. i.p. dose though weaker than codeine in 50 mg. kg. i.p. is stronger than mefenamic acid weight for weight (Table 10).

Work is in progress to confirm the analgesic activity of RH 8 by the oral route in mice. Analgesic activity of RH 8 in other species of laboratory animals like cats, guinea-pigs and rabbits is also being investigated.

TABLE 11—ANTIPYRETIC EFFECT

Dose 100 *mg./kg. oral*	No. of rats used	Total fall in temp. (sum of difference) °C.	Average fall in temp. (over a period of three hours) °C.
Mefenamic acid	9	27·1	3·0
Aspirin	3	9·5	3·2
Oxyphenbutazone	3	9·0	3·0
RH 1	3	7·6	2·5
RH 3	8	23·0	2·9
RH 9	3	13·5	4·5
RH 5	5	11·9	2·4
RH 7	7	9·9	1·4
RH 13	5	4·1	0·8
RH 8	23	69·2	3·0

Antipyretic Activity of Product Compounds

Since mefenamic acid is known to possess antipyretic activity also, it was thought desirable to investigate some of the compounds for their antipyretic effect (Table 11)

Antipyretic effect was studied in male albino rats weighing between 100 and 200 g. as described by Brownlee (1937). Eighteen hours before drug administration, the rats were injected subcutaneously with 15 per cent suspension of yeast in 2 per cent acacia gum in tap water saline. Drugs were administered orally. Rectal temperature was recorded with thermo-couple at the time of drug administration and thereafter hourly for three hours. Average fall in temperature over a period of three hours was calculated.

For purposes of comparison, aspirin and oxyphenbutazone were included in this study along with mefenamic acid.

Amongst the seven compounds investigated for their antipyretic effect, RH 9, *N*-(2,4-dichlorobenzyl) anthranilic acid was most potent, having an average fall of 4–5°C. RH 3 *N*-(*p*-chlorobenzyl) anthranilic acid and RH 8 compared favourably with mefenamic acid, aspirin and oxyphenbutazone in producing an average fall of 3°C., over a period of 3 hr in yeast fevered rats.

Anti-inflammatory Activity of Compound RH 8

As mefenamic acid has been claimed to be anti-inflammatory in addition to being analgesic and antipyretic, it was considered worthwhile to investigate the anti-inflammatory activity of RH 8.

TABLE 12—PERCENTAGE INHIBITION IN OEDEMA OF HIND PAWS OF RATS AT 1, 2 AND 3 hr AFTER SUBCUTANEOUS INJECTION OF 0·1 ml. OF 1% FORMALDEHYDE

Drugs (given 1 hr before formaldehyde injection) 100 *mg./kg. oral*	No. of rats used	1 hr	2 hr	3 hr
Oxyphenbutazone	24	17	27	27
Mefenamic acid	28	36	23	16
RH 8	34	36	38	28

Anti-inflammatory activity was estimated employing the method of Wilhelmi (1951), with our modification by measuring the volumes of mercury displaced by the oedematous hind paw of albino rats. Oedema was produced by subcutaneous injection of 0·1 ml. of an aqueous solution of 1% formaldehyde into the plantar surface of the hind paws of rats. Anti-inflammatory activity was assessed over a period of 3 hr by comparing the change in volumes of oedema produced in control animals and those treated with drugs administered orally one hour before formaldehyde injection into the paws. Percentage inhibition of oedema was calculated (Table 12).

RH 8 has been compared with oxyphenbutazone and mefenamic acid and it has been found to be stronger than oxyphenbutazone and mefenamic acid.

RH 8 is also being tested for anti-inflammatory activity in guinea-pigs by the ultraviolet erythema test and for the anti-granulation effect in rats.

Acute Toxicity of Compound RH 8

Acute toxicity of RH 8 was studied in mice by the i.p. route, as described by Turner. Groups of 10 mice consisting of an equal number of each sex were employed. Drug was injected in a suspension of 4% acacia gum in increasing dosage. The mice were observed for two hours after injection, then at the end of two hours, and later, daily for seven days. Approximate LD_{50} of RH 8 is 575 mg. kg. i.p. in mice. This compares very favourably with LD_{50} of mefenamic acid of 154 mg. kg. i.p. in rats as shown by Winder (Fig. 2).

Oral toxicity in mice and rats has also been studied and at a dosage of 2000 mg. kg., only 10 per cent deaths occurred. Hence it was not considered desirable to employ doses greater than 2000 mg. kg. and more so in view of the difficulty experienced in giving a thick pasty suspension of higher dosages.

On post-mortem examination of the dead animals congestion of lungs and liver was noted. Haemorrhages in stomach were also observed. Subacute toxicity studies in rats and dogs are in progress.

In view of the established fact that the most valid assessment of the analgesic activity of any substance is its efficacy in the relief of pathological

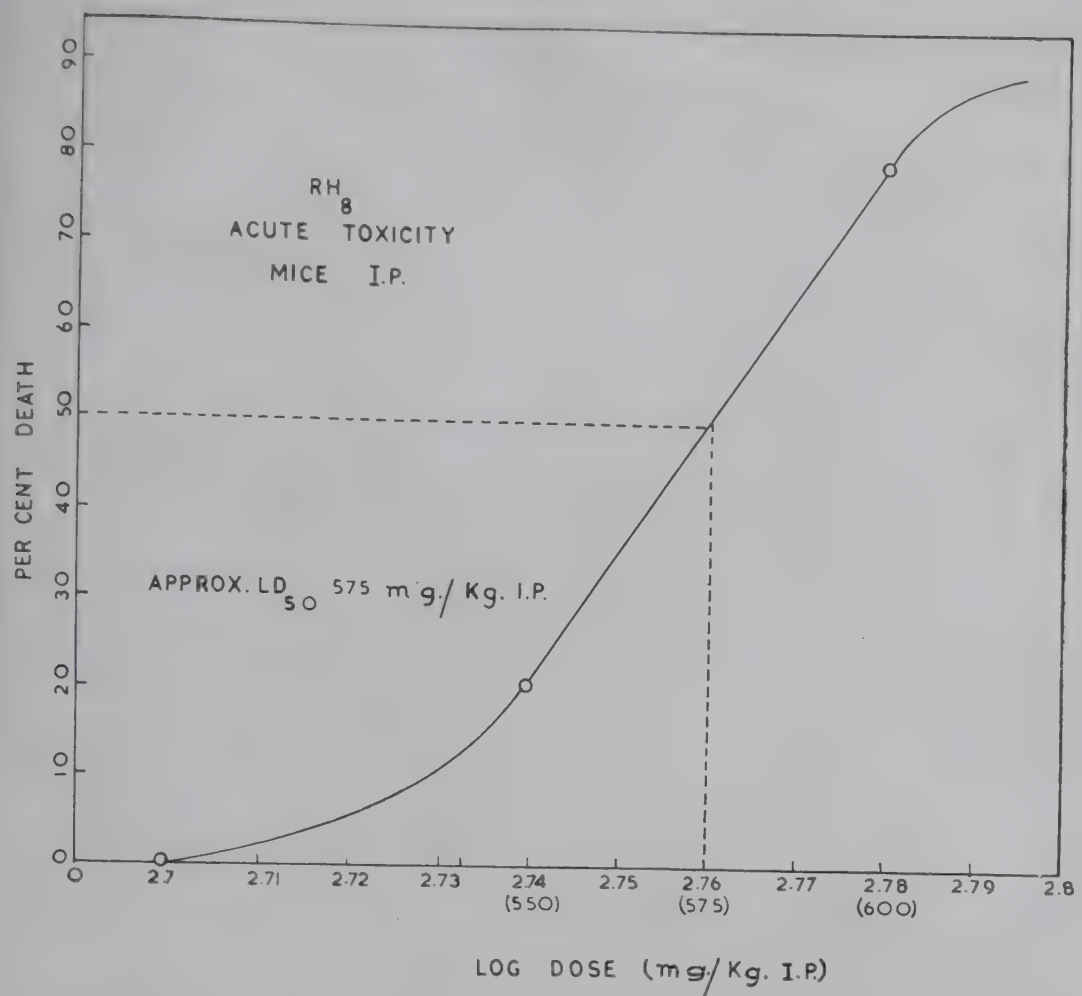


FIG. 2

pain, first clinical trials by the double blind method have been planned to be carried out as soon as the result of the subacute toxicity studies and further confirmatory evidence for analgesic effect in other species of animals by different routes of administration become available.

General Pharmacological Effects of Compound RH 8

RH 8 was also studied for its general pharmacology, a brief account of which is given here.

Dog blood pressure: In dosages up to 800 mg. there was no effect on blood pressure.

Isolated rabbit auricle: Up to 40 mg. there was no effect on the rate of amplitude; 50 mg. slowed the rate, decreased amplitude and at 200 mg. heart stopped temporarily, but recovered within 1–2 min. after washing.

Smooth muscle: Ten mg. produced a slight relaxation of guinea-pig ileum and slightly depressed 0·1 to 1·0 μg. of acetylcholine and histamine induced contractions.

Rabbit duodenum: Ten mg caused a little inhibition of normal contractions.

(Virgin) Rat uterus: Ten mg showed slight inhibition of spontaneous movements.

Frog rectus muscle (in eserinized ringer): Ten mg did not block contractions due to 10μg. of acetylcholine.

Frog sciatic nerve — Gastrocnemius muscle preparation: One mg did not show any curare-like action.

Rabbit eye: No surface anaesthesia and pupilary changes were noticed in 1% concentration.

Further neurological and behavioral studies and diuretic and metabolic studies have also been planned in collaboration with Dr U. K. Sheth, Professor of Pharmacology, G. S. Medical College, Bombay.

SUMMARY

A number of anthranilic acid derivatives have been screened for analgesic, antipyretic and anti-inflammatory activity. One of them, RH 8, is most potent as an analgesic and has powerful antipyretic and anti-inflammatory activities also. Further work on this compound is in progress.

ACKNOWLEDGEMENT

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REFERENCES

1. Brownlee, *J. Pharm. Pharmac.*, **10** (1937), 609.
2. Cass, L. J. & Frederik, S. F., *J. Pharmac. exp. Ther.*, **139** (1963), 172.
3. Kendall, P. H., *Br. med. J.*, **1** (1964), 117.
4. Turner, R. A., Screening Methods in Pharmacology, (1965 ed.) p 302, Academic Press, New York.
5. Wilhelmi, G. & Domenjoz, R., *Arzneimittel Forsch*, **1** (1951), 151.
6. Winder, C. V., Wax, J., Scotti, L., Scherrer, R. A., Jones, E. M. & Short, J. W., *J. Pharmac. exp. Ther.*, **138** (1962), 1195.

Peripheral and Central Actions of *Areca catechu*

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The first introduction of areca nuts as a component of the masticatory mixture is shrouded in mystery. It has been mentioned in Aryan civilization and forms part of the ritual offerings to Gods and is also described in the Ayurvedic treatises[1–7].

Therapeutic usage of the nuts in the olden days can be gauged by the meticulous description given of the slightly varying properties of the nuts obtained from different parts of India and also to the specific properties attributed to the nuts during different periods of maturity and when subjected to treatment like drying, boiling, etc.

The types of nuts to be used, the method of collection and the toxic effects induced by large doses are vividly presented. Among the toxic effects mentioned are tremors, perspiration, an appearance of darkness, excessive salivation, giddiness, loss of voluntary control, exhaustion and loss of consciousness.

Although *Areca catechu* is no longer an official drug, the reputation it enjoys is reflected in its use by millions of people even today. In the history of therapeutics, it is not uncommon to find plant products considered to be efficacious in the ancient systems of medicine but discarded under the later 'scientific system' as not substantiating their therapeutic claims, being rediscovered with further advances in our basic knowledge and experimental techniques.

The natural products which have been largely in use since ancient times have revealed that majority of such products exhibit effects on the higher nervous system either as general stimulants, tranquillizers, hallucinogens or analgesics. The classical examples are cocaine, opium, hashish and, to a lesser degree, tobacco, coffee and tea. The use of areca nut could be classified as coming under the latter group. Many are the conditions in which the nuts are considered to be therapeutically useful. Giving sereneness

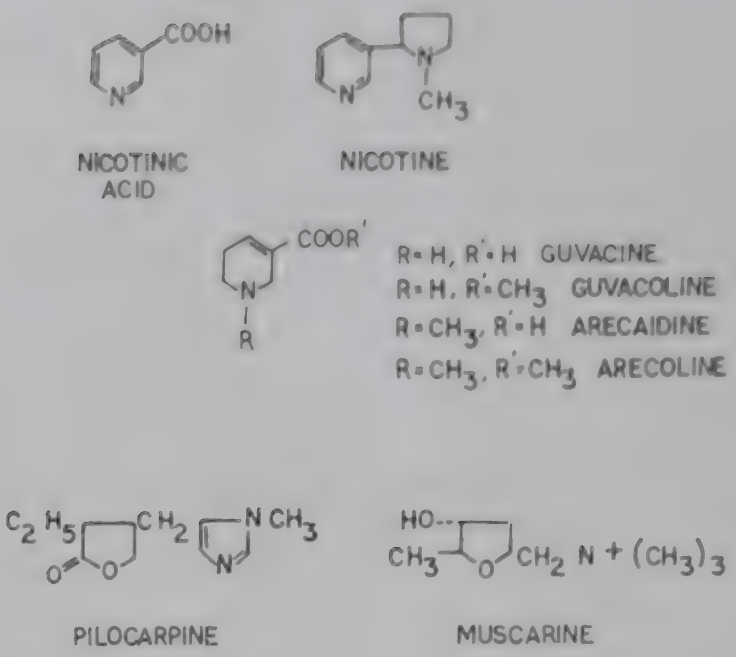

FIG. 1 — STRUCTURE ALKALOIDS OF *Areca catechu*

and calmness to the individual, endowing increased strength of mind to carry out difficult tasks and enhancing the powers of concentration are some of the effects on the higher nervous system claimed for the nut besides its influence on the peripheral autonomic nervous system in increasing the secretions of digestive juices and the power of digestion. Habit formation and appearance of withdrawal symptoms lend evidence to the suggested influence of the nut on the central nervous system.

Betel chewing is a passion for millions of men and women and once it becomes a habit, it is almost impossible to stop it. The alkaloids which are considered to be the active ingredients of the nut are β-substituted pyridine derivatives related to tetrahydronicotinic acid (Fig. 1).

These alkaloids show structural or biogenetic relationship to nicotine but their pharmacological effects, however, are partly different[5].

The alkaloids isolated from areca nut are arecoline which constitutes the main alkaloidal component; arecaidine and guvacine in smaller quantities, isoguvacine in traces and arecolidine and guvacoline in very minute quantities. No detailed systematic pharmacological study of these alkaloids has been carried out and very little is known of the toxicology, metabolism and inter-relationship of the pharmacological effects of these components.

Even with arecoline, the one alkaloid of areca which has attracted the attention of chemists, pharmacologists and clinicians, information pertaining to its mode of action, metabolism and its influence on functions of the CNS are far from complete.

In all respects arecoline is qualitatively similar in its muscarinic action to pilocarpine[6]. But there is no apparent chemical basis for the similarity

in the actions of arecoline, pilocarpine and muscarine. Arecoline also possesses nicotine like properties as manifested by its stimulation of the skeletal muscle, a rise in blood pressure after atropinization and stimulation of the carotid body. The nicotine like actions are to be expected on the basis of the piperidine structure which probably accounts for the euphoric action of the betel nut[9].

Arecoline is thus a cholinergic agent with both muscarinic and nicotinic actions though the former is more prominent. This alkaloid has no structural relationship with acetylcholine, the neurotransmitter of the parasympathetic autonomic nervous system. Very little is known about the site and mode of action of arecoline excepting that the effects are cholinergic.

Arecoline is classified as a parasympathomimetic drug and its peripheral effects are considered to be muscarinic in character. Though inhibited by atropine, its cholinergic action is in many respects qualitatively dissimilar to acetylcholine. An investigation of the nature of atropine antagonism on rat ileum has revealed the inhibition to be one of non-competitive antagonism while atropine and acetylcholine are competitive inhibitors.

Atropine Antagonism to Arecoline on Rat Ileum

Atropine appears to be a non-competitive antagonist to arecoline on the rat ileum, since the degree of antagonism is determined by the concentrations of the antagonist only, and the antagonistic action is insurmountable. There is a gradual decline and disappearance of the effect of arecoline at high concentrations of the atropine (Fig. 2).

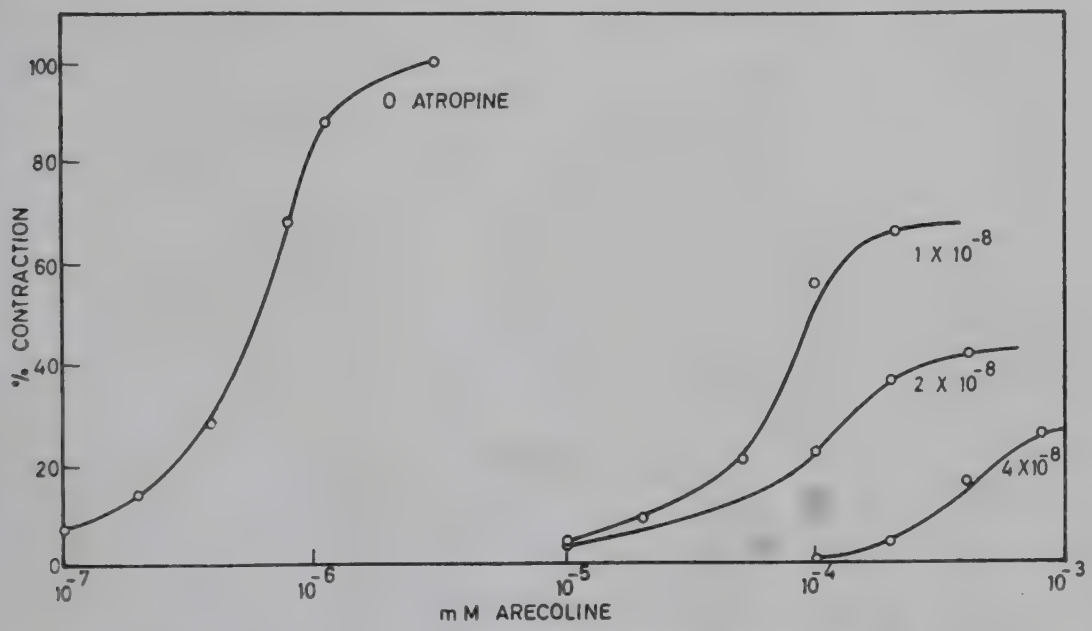

FIG. 2 — CUMULATIVE LOG-CONCENTRATION RESPONSE CURVES FOR ARECOLINE IN PRESENCE OF ATROPINE (note decrease of the maximal height and the slope of the curves)

Cumulative log-concentration response curves for the action of the parasympathomimetic arecoline in the presence of various concentrations of the parasympatholytic atropine are shown in Fig. 7. Besides a decrease of the maximal height and the slope of the curves, a shift in the curves also takes place.

The shift caused by atropine in the log dose response curve for arecoline in rat ileum is not based on a possible competitive inhibiting action of atropine. On the basis of the dose response curve, one may conclude that there is a non-competitive antagonism between atropine and arecoline. This type of curve may also be obtained when the specific receptors for the agonist are occupied in an irreversible way by the antagonist. Since the antagonist, atropine in this case, can be easily washed away from the tissues, an irreversible binding is highly improbable and irreversible blockage can thus be eliminated.

Atropine is a competitive antagonist for acetylcholine on the isolated gut while acting as a non-competitive antagonist in higher concentrations on rectus abdominus muscle of the frog. This points to differences between the receptors for acetylcholinomimetics in the various tissues in different species. Though not proved by scientifically controlled experiments, areca nut can be considered to be psychopharmacological agent, since, as mentioned earlier, its use can be likened to the use of other habit forming, centrally acting drugs. In fact the regular habit of chewing the areca nuts seems to replace the use of alcoholic beverages in such people.

Arecoline appears to be one of the constituents involved in the psychopharmacological effects. It is now used as a pharmacological tool to map out the regions of the brain containing the muscarinic type of receptors in the cholino reactive system and to differentiate the actions of antiparasympathomimetic drugs[10,11].

The existence of cholinoreactive systems in the brain has been well established by these studies. In an analysis of the specificity of the receptors of this system in various regions of the brain, it is observed that distribution of qualitatively different choline reactive structures is far from being uniform.

Cholinoreactive systems of the reticular formation exhibit certain degree of selectivity to the stimulating or the blocking drugs. While acetylcholine produces distinct stimulation of both the sensory and motor region of the cortex and nicotine causes greater changes in the electrocorticogram than in the bioelectrical activity of the reticular formation, arecoline, which imitates the muscarinic action of acetylcholine specifically induces greater bioelectrical changes in the reticular formation. This arecoline activity is abolished by benactyzine. Thus arecoline and its antagonists have been found to effect cholinoreactive structures of the mid-brain reticular formation to a greater extent than those of the cerebral cortex[10].

In the reticular formation of the mid-brain, arecoline sensitive structures evidently predominate.

It is possible to say that cholinoreactive structures of the reticular formations of the brain take part in producing the 'arousal effect' in the cortex, and that the transmission of the impulses between these parts of the brain is mainly effected through cholinergic synapses.

By influencing the cholinoreactive structures of the brain the course of conditional or unconditional reflexes can be changed[10].

Arecoline, by its influence on the cholinergic system and particularly on the cholinoreactive sites of the reticular system can thus be expected to produce arousal behaviour and also affect the response to reflexes, conditioned or unconditioned.

Besides arecoline, the areca nut contains various other alkaloids which, though present in small quantities, are definitely likely to influence the actions of arecoline. No systematic detailed investigation on the effects of these alkaloids individually and in the presence of arecoline has been carried out so far.

While alkaloids do constitute the main active ingredients of the nuts, the pharmacological properties of the non-alkaloidal components cannot be ignored. Our recent studies have shown that such fractions do exhibit powerful biological actions on microorganisms[12] and on isolated organs of the body[13].

Tannins of Areca catechu

An analysis of the areca nut reveals that 'tannins' (polyphenols) form an appreciable fraction of the constituents of the areca nut, ranging from 8 to 15 per cent. The major portion of areca tannins are of the condensed type. Besides the polymerized flavan 3 : 4 diols and catechin, monomeric flavan 3 : 4 diol (leucocyandin) and catechin have also been identified in the polyphenolic constituents of the areca nut[14,15]. In addition to these, the ethyl acetate extract of the nut has shown the presence of three more phenolics in the chromatogram which remain to be identified[16].

These types of flavonoid compounds occur in many foodstuffs of plant origin[17]. Flavonoids, in general, are known to exhibit various biological activities such as antimicrobial action[18,19], antioxidant properties[20], enhancement of capillary resistance[21], vasoconstrictor action[22], reduction of capillary permeability[23] and potentiation of the actions of naturally occurring amines in the body[24].

The crude extracts of *A. catechu* have been shown in our earlier studies to be active vasoconstrictors and potentiators of adrenaline action on capillary circulation[25,26]. The tannins of areca exhibit antifungal and antibactericidal properties[12] and are spasmogens of some smooth muscles[13].

The results of the investigations on some pharmacological properties of the tannins of areca are detailed below.

The tannins were extracted from ripe nuts obtained from a plantation in Kerala, using lead acetate extraction procedures.

The influence of tannins on some smooth muscle preparations and on the spasmogenic action of the alkaloid of the nut arecoline and on acetylcholine was studied by the standard Schultz-Dale technique.

RESULTS

Ileum (rat). At low concentrations (1 γ/ml.), tannin sensitizes the strip to acetylcholine without itself producing any direct stimulatory action. At 10 γ/ml. the drug shows slight contraction and potentiation of acetylcholine action (Plate Ia). No such sensitization was observed for barium contraction. The contractile effect of 5-HT was also not influenced by the presence of tannin.

Ileum (rabbit). As with the rat ileum, tannin exhibits spasmogenic action and enhances the amplitude of spontaneous contractions. Both these effects are neutralized by atropine. Recovery, after removal of the drug from the bath is fairly quick (Plate Ib).

Bladder (rabbit). The influence of tannin on arecoline action was studied in this experiment. Slow contraction of the musculature at 10 γ/ml. and an increased spasmogenic action at 50 γ/ml. can be seen.

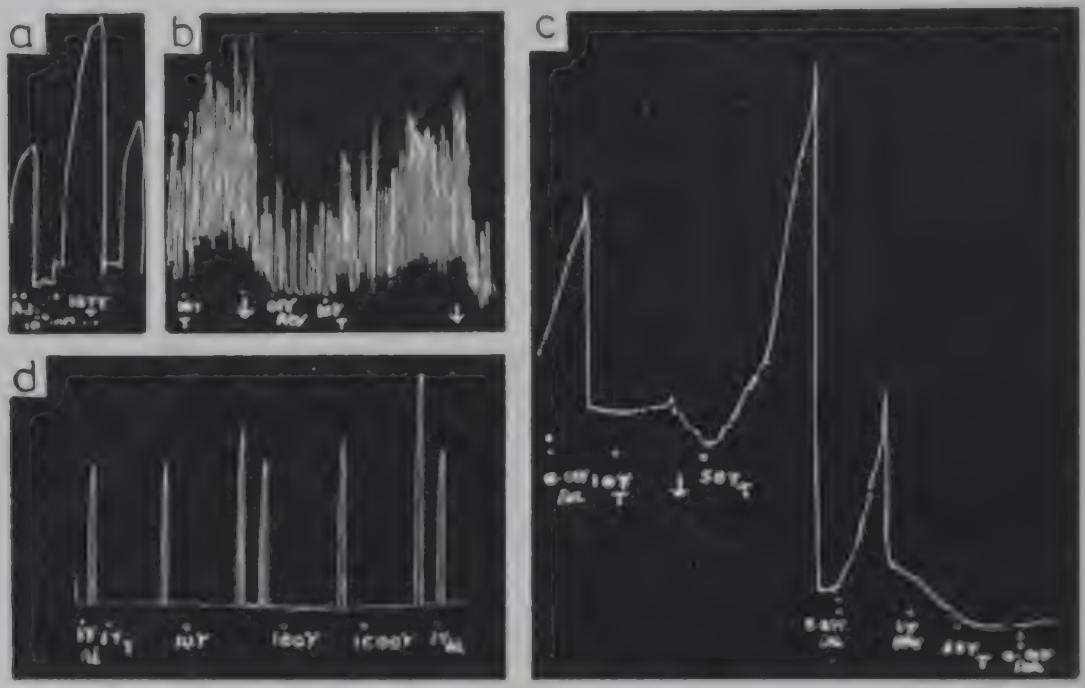

PLATE I — (a) RAT ILEUM (Ach. effect; potentiation in presence of 10γ/ml. of tannin of areca seen). (b) RABBIT ILEUM (Neutralization of tannin effect by atropine is seen); (c) RABBIT BLADDER (Tannin potentiates the arecoline contraction). (d) RAT SEMINAL VESICLE (Tannin sensitizes the tissue to noradrenalin).

Arecoline effect was potentiated. Atropine, as on ileum, neutralized the effect of both tannin and arecoline (Plate 1c).

Seminal vesicle (rat). Potentiation of noradrenalin contraction can be seen in this experiment. Tannin exhibited no contractile action on this organ even at 1 mg./ml. (Plate 1d).

Rat uterus. The effect of tannin was one of sensitization to acetylcholine in low concentration and a depression at higher levels.

Frog rectus. Tannin showed no spasmogenic effect on this musculature. The contraction induced by potassium chloride was unchanged in presence of tannin.

In general, it can be concluded that areca tannin is a potentiator of the action of acetylcholine on ileum and uterus of the rat as also of noradrenalin on rat seminal vesicle at low concentrations. At higher concentrations, the drug is a spasmogen of ileum but not of uterus or seminal vesicle and depresses the contractions induced by ache and noradrenalin.

Acute Toxicity of Areca Extracts and their Influence on the Motor Activity of Mice

Alcoholic extracts of the nut which contained the alkaloids and the polyphenols, ethylacetate fraction showing the presence of only the polyphenols and the alkaloid arecoline hydrochloride were used in these studies.

Gross observations on the motility and development of analgesia were made on mice, to which were administered varying doses of the drugs by different routes. The results are summarized in Table 1.

Arecoline, in doses of 1–2 mg./kg. given s.c. to rats is reported to cause motor restlessness and marked analgesic effect and that atropine abolishes these effects[27].

In our studies on mice, it was observed that arecoline hydrochloride 0·4 mg./kg. given s.c. caused no motor restlessness but induced a slight degree of sedation lasting for about 15 min. Four mg./kg. given s.c., induced increased sedation. The animals were found to be inactive with absence of the usual probing movements. This inactive period was not observed after atropine administration (4 mg./kg.; s.c.). Analgesic effect was also observed. These effects lasted for about 20 min. At 20 mg./kg., the mice adopted a hunch-back appearance. Inactivity persisted for nearly half an hour. Salivation was present. Normalcy was reached after about two hours. These symptoms were more prominent at 40 mg./kg. An immediate appearance of tremors all over the body, hunch-back, salivation, respiratory distress and inactivity were the symptoms seen.

Arecoline, 0·4 mg./kg. s.c. was non-fatal while with 40 mg./kg., more than 50 per cent mortality was seen.

Arecoline, 0·5 mg./kg., given i.v. produced an initial increased activity followed by tremors. These effects were more prominent with 1·0 mg./kg.

TABLE 1—ARECA EXTRACTS AND ACUTE TOXICITY

Compound	Dose *mg./kg.*	Route	Activity of mice	Analgesia	Other symptoms
Alcoholic extract	40	s.c.	Inactive	+	Salivation
Ethyl acetate extract	40	s.c.	—	—	..
	40	i.p.	—	—	..
	200	i.p.	Inactive	—	..
	400	i.p.	Inactive	—	Death
	40	i.v.	Increased activity	—	..
	200	i.v.			Death
Arecoline hydrochloride	0·4	s.c.	Slight sedation	+	..
	4	s.c.	Slight sedation	+	..
	20	s.c.	Inactive	+	Salivation
	40	s.c.	Inactive		Salivation, tremors, respiratory distress
	0·75	i.p.	Slight sedation	—	
	1·0	i.p.	Sedation	+	
	0·5	i.v.	—	+	Slight tremors
	1·0	i.v.	Immediate enhanced activity later sedation		Tremors
	2·0	i.v.			Death

s.c., subcutaneous i.p., intraperitoneal i.v., intravenous
— no effect + effect seen

After these initial stimulatory effects, a period of depression was observed which ranged from 5 to 20 min. depending on the dose administered. Two mg./kg. i.v. was fatal to all mice.

The effect of total alcoholic extract was similar to those of arecoline.

Ethyl acetate fraction, which consisted mostly of the polyphenols, at 40 mg./kg., intravenously was a stimulant, enhancing the active move-

ments of the animal. This activity was not seen after subcutaneous injection. Higher doses above 150 mg./kg. were lethal to the animals.

Our results indicate that the alkaloid arecoline and the crude alcohol extract given subcutaneously depress the general activity, without any initial stimulation. The increased motor restlessness reported in rats after arecoline administration s.c. was not seen in our studies with mice. But a transitory increased activity could be observed after i.v. administration of arecoline and ethyl acetate fractions.

In presence of atropine, the sedative action of arecoline was not seen. But an interesting observation that needs further confirmation was the enhancement of arecoline sedation after prior administration, subcutaneously, of ethyl acetate fraction while the latter fraction by itself was a stimulant of the motor activity when given i.v.

Areca nut is thus seen to contain components of varying pharmacological actions like stimulation and sedation. The final effect of the chew which will contain varying amounts of all the principles is not merely that of the alkaloids but will depend on the quantitative aspects of the individual components as also on the nature of the interactions between the components.

The route of administration of the drug also appears to be important in the manifestation of the symptoms. The sedative effect predominates when arecoline is given subcutaneously and intraperitoneally. The absence of stimulation by this route may be due to the slow absorption and of inability to reach the requisite concentration or the sedative action may be the effect of the metabolic products of arecoline. This may also explain the sedative effect seen after the initial stimulation induced by the intravenous administration of the drug.

Arecoline Induced Changes of Higher Nervous Activity

The term 'higher nervous activity' has been applied to both spontaneous changes of behaviour (e.g. orientation activity) and the conditioned reflex by Pavlov.

Various methods are in vogue for studying the effect of drugs on spontaneous activity. The method recommended by Lat appears to be suitable for determining the excitatory activity on the CNS and its modification by psychotropic drugs, using rats as experimental animals. The method adopted was similar to the one described by Votava *et al.*[28]. Adult male rats kept in standard condition were used. Orientation activity was investigated in a wooden chamber ($46 \times 27 \times 39$ cm.) which was divided by a barrier into two parts 16 and 30 cm. long, respectively. The barrier could be passed through a door of 17×15 cm., which could be opened by pulling it upwards. The rat was placed in the smaller chamber with the door closed for a specified period. After that time the door was opened so that the rat could pass into the larger chamber and expose the whole box for another specific period.

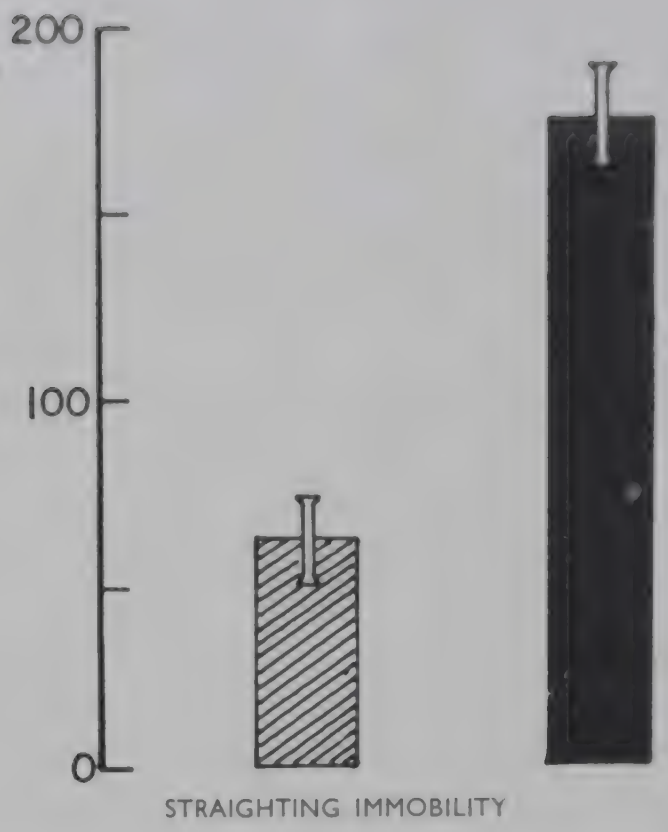

FIG. 3—INFLUENCE OF ARECOLINE ON ORIENTATING REACTION IN RATS

The behaviours recorded were the ambulation period, rearing, the number of times the rat passes through the door and general activity.

The test substances were administered in the doses required and test conducted immediately.

A group of 5 animals were used for each test dose and the averages recorded.

Arecoline hydrochloride was given i.v. 1 mg. kg. At this dose tremors all over the body were seen which lasted for 1½ to 2 min. The animals were then kept in the wooden chamber and the reactions noted over a period of 10 min. Saline given i.v. served as control.

The effect of arecoline is shown in Fig. 3.

It is seen that the drug prolonged the duration of immobility and reduced rearing movements. In general the animals were found to be inactive and recovered normalcy only after a period of 45 min. to 1 hr.

With doses of arecoline where only mild tremor or none was observed, the same type of reactions were seen excepting that they recovered normalcy in half hour—some animals showed a slight increase in activity for the first 1 or 2 min. and this was followed by the prolonged inactive period.

The disturbance in the coordination of the movements, 10 min. after arecoline administration was studied by the 'Rota rod test'. This is a very

suitable technique for observing quick orientation. In general, the method was similar to the one observed by Tripod *et al.* Five mice separated by barriers are placed on the rod which rotates eight times per minute. The inability of the mice to hold its position within the two minutes of observation period was considered to indicate the positive effect of the drug. The period required to regain normalcy was observed by testing the animal on the rota rod every 15 min. Arecoline treated mice which appeared sedated after the initial tremors were unable to maintain their position on the rod for about 30 min. after which period normalcy was regained.

Though the technique appears simple, the interpretation needs caution since central inhibition (narcosis), central stimulation (excitation and convulsions) and muscle relaxation (curare like effect) might induce the dropping off of the mice. No curare like effects were seen after arecoline and the initial tremors had completely passed off at the time of the test. Hence it is very likely that the observed effect is due to the central inhibition by arecoline.

The influence of arecoline in smaller doses over a prolonged period is under study.

Monoamineoxidase Inhibitory Activity of Arecoline and Polyphenols of A. catechu

Since the discovery of monoamineoxidase (MAO), many types of chemicals have been found to be inhibitors of this enzyme. A partial list of inhibitors includes aliphatic alcohols, amidines, guanidines, isothioureas, xanthines, methylene blue and choline phenyl ethers. A number of widely used drugs such as ephedrine, amphetamine, local anaesthetics and antihistamines also have been shown to inhibit MAO *in vitro*. Most, if not all of these compounds, however, appear to be essentially devoid of *in vivo* inhibitory activity[29].

The intense research, as a result of the clinical efficacy of MAO inhibitors as antidepressants and toxicity of hydrazine derivatives so far in use, has focussed attention on the MAO inhibitors of non-hydrazine group.

The first non-hydrazine compounds found to have potent *in vivo* MAO inhibitory activity were the harmala alkaloids harmine, harmaline and related carboline derivatives[30]. These alkaloids exhibit hypotensive and central excitatory action but their other toxic properties preclude their use in man.

Areca catechu nuts which are used commonly in masticatory mixtures are thought to produce euphoria and induce mild stimulation. Our studies indicate arecoline and ethyl acetate fractions of the areca nut given i.v. to be initial stimulants of CNS.

Arecoline and the polyphenols of the areca nuts have also been shown to potentiate the adrenaline activity. Since the stimulant activity on the CNS and potentiation of epinephrine action are generally considered to

reflect the activity of MAO inhibitors, a study of MAO inhibitory activity of arecoline and the polyphenols of *Areca catechu* has been carried out.

Evaluation of MAO inhibition. MAO activity of rat brain and liver homogenates was measured by the method of Sporderma *et al.*[31], using 4×10^{-3}M. serotonin as the substrate. All inhibitors were preincubated for 15 min. with the enzymes before the addition of the substrate.

MATERIALS AND METHODS

Male rats weighing between 250 and 300 g. were sacrificed by decapitation. Brain and liver were removed immediately; blotted to remove excess of blood, chilled and homogenized with water such that it contained 37·50 mg. ml. protein in brain homogenate and 72·50 mg. ml. protein in liver homogenate. Incubation mixtures contained 1 ml. of homogenate, 0·3 ml. phosphate buffer 0·5M.; *p*H 6·9.

Phosphate buffer. Solutions consist of gram mole of each of (a) K_2HPO_4, *p*H 9·4; (b) KH_2PO_4, *p*H 4·5. *p*H 7·0, 0·5 M. phosphate buffer consists of 4·0 ml. of KH_2PO_4 and 6·0 ml. of K_2HPO_4 solution diluted with 10 ml. of water (total volume 20 ml.).

Substrate 5-hydroxytryptamine (5-HT) 4×10^{-3}M. in a final volume of 3 ml. was made up with water. It was then incubated at 37° C. in a metabolic shaking incubator for 30 min. For inhibitors, a pre-incubation of 15 min. with the enzyme was done before the addition of the substrate and incubation. Other experiments included controls which lacked either enzyme or substrate. Enzyme activity was measured by the disappearance of substrate.

5-HT is measured colorimetrically by the method of Udenfriend *et al.*[32].

In vivo method.[33] The analysis of MAO activity of tissues from animals pretreated with MAO inhibitors is considered as '*in vivo*' activity.

In vivo studies with MAO inhibitors. The inhibitors studied were: (a) Tranylcypromine (Parnate) 40 mg. kg. i.v.; (b) Arecoline 1 mg. kg. i.v.

Male rats weighing between 150 and 180 g. were used. A group of four rats were used for each drug. Animals were sacrificed 18 hr after injection. The brains were removed quickly and homogenates made so as to contain approximately 275 mg. ml. Protein estimation showed 37·50 mg. ml. of protein in the homogenate and 1 ml. each was taken for the test. The rest of the procedures adopted were similar to the *in vitro* enzyme estimation. Untreated rat brains were similarly homogenized and used as controls.

RESULTS

In vitro activity of the compounds is shown in Table 2. The *in vivo* inhibitory effect of the drugs on the MAO activity is shown in Table 3.

TABLE 2—*IN VITRO* MONOAMINEOXIDASE INHIBITORY ACTIVITY OF ARECOLINE, POLYPHENOLS OF *A. catechu* AND OTHER MAO INHIBITORS

Compounds	Source: Brain		Source: Liver	
	Conc.	Inhibition, %	Conc.	Inhibition %
Iproniazid sulphate	$8{\cdot}7 \times 10^{-4}$M.	50	$3{\cdot}0 \times 10^{-4}$M.	50
Tranylcypromine	$3{\cdot}3 \times 10^{-4}$M.	100	$1{\cdot}0 \times 10^{-4}$M.	100
Acetylcholine hydrochloride	$2{\cdot}75 \times 10^{-2}$M.	20	$5{\cdot}5 \times 10^{-2}$M.	No inhibition
Nicotine hydrogen tartrate	$3{\cdot}33 \times 10^{-3}$M.	100	$6{\cdot}67 \times 10^{-3}$M.	83
Tannin from *Areca catechu*	5 mg.	5	..	..
Leucoanthocyanidin	5 mg.	16·8	5 mg.	4·2
	10 mg.	33·6	10 mg.	26·3
Arecoline	$2{\cdot}1 \times 10^{-2}$M.	40	$4{\cdot}0 \times 10^{-2}$M.	30
			$8{\cdot}0 \times 10^{-2}$M.	56

TABLE 3—*IN VIVO* MONOAMINEOXIDASE ACTIVITY OF MOUSE BRAIN AFTER PRETREATMENT WITH ARECOLINE AND TRANYLCYPROMINE

	In vivo activity of MAO inhibitors	
	Dose	Inhibition, %
Tranylcypromine	40 mg./kg intravenously	100
Arecoline	1 mg./kg intravenously	32·3

Relative Potencies of the Various Types of Inhibitors

The observed relative potencies of MAO inhibitors depend on the test procedure, species, tissue, route of administration of the drug, etc.

There are very few studies designed to obtain the relative efficacy of MAO inhibitors. We have compared the inhibitors *in vivo* by measuring the influence on MAO activity of the tissues 18 hr after administration of the compound.

In vitro MAO activity of brain tissue is seen to be completely inhibited by tranylcypromine at $3{\cdot}3 \times 10^{-4}$M. concentration. Iproniazid is also fairly active in 10^{-4}M. level. Nicotine at a slightly higher concentration has shown

100 per cent inhibition. The activity of acetylcholine and of arecoline are of still lower order. Since leucoanthocyanidin and tannins of areca nut were crude materials molar concentrations could not be mentioned but their activity also seems to be low.

On the liver MAO activity also, the same trend of inhibition is seen. Arecoline is more inhibitory than acetylcholine. The selective activity of tranylcypromine in blocking brain MAO more than liver reported in mice and rabbits is also seen in rats[30,31].

The preliminary studies on *in vivo* effect of arecoline indicate that even after 18 hr there is a fair degree of inhibition in the brain of arecoline treated mice while tranylcypromine has shown complete absence of enzyme activity as expected. Since arecoline effect may be more rapid, it is essential that 'time-activity' relationships are studied in detail.

Thymoleptic activity of arecoline. Since arecoline has a special predilection to the cholinergic receptors in the mid-brain reticular system and induces 'arousal reaction', the thymoleptic (antidepressant) activity of the drug was studied. Most of the known thymoleptic agents are inhibitors of monoamineoxidase and arecoline is also found to be a weak MAO inhibitor.

One of the screening methods for thymoleptic or antidepressant action is the 'antagonism to reserpine'[33].

Reserpine, 0·75 mg. kg. i.p. produced only a transient sedation and ptosis. With 1 mg. kg. i.p. the sedation and full ptosis were seen.

For the test, arecoline hydrochloride 1 mg. kg. i.p. was given and was followed by reserpine 1 mg. kg. i.p., an hour later. Rats of similar age and sex given only reserpine served as controls. The degree of sedation and ptosis was recorded at intervals. Iproniazid 50 mg. kg. i.p., given 18 hr earlier, served as a positive control of an MAO inhibitor.

Iproniazid treated animals were fully active and exhibited no sedation or ptosis. In arecoline treated rats, the ptosis was less and general activity slightly more than those given reserpine only.

Arecoline at the dose given appears to be a mild antagonist of reserpine.

When reserpine effect was fully developed, arecoline even at double the above dosage exhibited no antagonistic action.

Areca catechu and sleeping time in mice. The effect on the hypnotic action of anaesthetics is one of the important and instructive measures of the action of drugs which act on the central nervous system. Barbiturates are the most preferred anaesthetics.

Intravenous thiopental in mice with the dose suitably adjusted has given consistently uniform results. With other routes and other barbitals the variations were greater.

Thiopental 40 mg. kg. i.v., the recommended dose in the literature[34], was found to be fatal to our strain of mice. The same dose given i.p. produced hypnosis ranging from 2 hr 8 min. to 4 hr 12 min. But the time taken for

TABLE 4—INFLUENCE OF CHLORPROMAZINE, ARECOLINE AND TANNINS OF *A. catechu* ON THIOPENTAL HYPNOSIS IN MICE

(Route: intravenously)

Drug		Sleeping time *min.*
Thiopental	..	2·47
do	*Chlorpromazine	24·75
do	*Arecoline	2·8
do	*Ethyl acetate extract of *A. catechu*	2·7
do	*Tannin	3·86
do	*Chlorpromazine tannin	41·5

*Drugs given ½ hr prior to thiopental

the hypnosis to develop varied considerably. Twenty mg./kg. i.v. was found to be optimum dose for inducing hypnosis which developed almost immediately and lasted from 2 hr 30 min. to 4 hr 30 min.

Male mice weighing 18 to 25 gm. were given the required dose of the drug i.v. and half an hour later thiopental 20 mg./kg. was given by the same route. The time taken for the animal to right itself was taken as the end point of hypnosis. The drugs tried included arecoline, the ethyl extract of *Areca catechu*, tannins of the nut and chlorpromazine.

Chlorpromazine at 1 mg./kg. i.v. enhanced the sleeping time. Arecoline hydrochloride, 0·2 mg./kg. i.v. was a very slight potentiator of thiopental hypnosis. Ethyl acetate extract had no influence on the sleeping time while tannin 8 mg./kg. besides enhancing the thiopental action also potentiated further, the chlorpromazine effect when given concomitantly (Table 4).

DISCUSSION

The interpretation of the results obtained is rather ambiguous because the alteration in the time of sleep may be the result of either of the effect on central nervous action—as it is in the case of phenothiazine derivatives or reserpine or of the modification of the barbiturate metabolism as with SKF 525 A.

The influence of the drug on the hypnosis induced by the barbiturates also depends both on the type of barbiturate used and the species of animal experimented. Methylphenidate, e.g. antagonizes the hypnotic effects of thiopental in rats but exhibits no influence on barbital or phenobarbital-induced sleep[35].

The mode of action involved is difficult to analyse since enhancement or antagonism of hypnosis can be effected by various means and is not limited to tranquillizing drugs only.

The antidepressant drugs potentiate the effects of barbiturates[38]. Nicotine is among the drugs producing an insignificant increase in barbiturate-induced sleeping time[36].

Sleep produced by the electrical stimulation of the preoptic area of the hypothalamus or local injection of acetylcholine can be neutralized by stimulation of reticular formation[7]. Cholinergic substances injected into parts of the limbic brain regions are effective in inducing sleep. In other regions they cause arousal. Barbiturates are considered to exert their hypnotic action via the block of the reticular formation[38].

Arecoline with its faint MAO inhibitory activity and arousal reaction as a result of its specific stimulating action on the cholinergic receptors in the reticular formation could normally be expected to antagonize the sleeping time. Whether arecoline affects other cholinergic sleep-inducing areas thus counteracting its influence on reticular formation or other mechanisms are involved remains to be elucidated.

Since many environmental factors are involved in hypnotic action particularly the dose of the drug and the route of administration, it is difficult to correlate the experimental results with clinical findings.

Arecoline Tremors

Tremor-like movements occur after administration of a great variety of drugs. Di-isopropylfluorophosphate, eserine, decamethonium, 5-hydroxytryptamine, pilocarpine, tubocurarine, and mepasulphate induce tremors after intraventricular injection[39].

Tremors have been observed in mice after intraperitoneal injection of tremorine, nicotine, amino alcohol, harmine and lysergic acid-diethylamide[10] and 5-HTP+iproniazid.

Tremor is one of the more characteristic effects of nicotine. Although nicotine has a demonstrable effect directly on skeletal muscles, nicotine tremor is undoubtedly a result of central nervous system stimulation[11].

Acetylcholine too may be involved in the regulation of the tremor since anti-cholinesterases produce tremor[39].

Since the effects of arecoline are predominantly para-sympathomimetic and closely resemble those of pilocarpine which produces tremors, the influence of this alkaloid on tremor induction has been studied.

Various methods are employed for studying the tremerogenic action. These comprise from visual observation to recording the movements with electronic equipment for quantitation. For our studies varying amounts of arecoline were introduced by different routes and the extent and duration of tremors induced were recorded by direct visual observation.

Dosage for the production of tremor depends on experimental condi-

tions, more particularly rate and route of administration. It is also important whether tremor is studied as an end in itself using tremor subconculsive doses or whether tremor is merely recorded as a passing phase on the road to convulsions produced by convulsive doses of the drug.

In mice, arecoline hydrochloride 30 mg./kg. orally, produced tremors after an interval of about 15 min. At this dose peripheral parasympathetic actions were also visible.

Subcutaneously, tremors were seen at 8 mg./kg. which persisted for about 15 min.

Intravenously 0·4 mg./kg. produced slight tremors. This was manifested to a greater extent at 1 mg./kg. The tremors induced at these dosages were not followed by convulsions.

Influence of cholinolytics and other centrally acting drugs on arecoline tremors. It is now well proved that cholinergic mechanisms do play a prominent role in the conduction of nerve impulses in the CNS. Hence, it may be expected that drugs which are parasympathomimetic in action would influence the effect of psychotropic drugs and vice versa. Therefore, it was thought useful to study the effect of MAO inhibitors, central and peripheral cholinolytics, and drugs which modify the peripheral actions of arecoline like areca tannins and thiamine on arecoline tremors which result from its central stimulatory activity.

Monoamineoxidase (MAO) inhibitors. Hydrazine derivatives have been found to interact with a variety of pharmacological agents resulting in an alteration of their responses[33]. They are effective anticonvulsants against electroshock and metrazol seizures.

The following MAO inhibitors were tried for their influence on arecoline tremors: (1) phenalzine sulphate 40 mg./kg. i.v.; (2) isocarboxazide 80 mg./kg. i.v.; (3) iproniazid 40 mg./kg. i.v.; (4) nialamide 40 mg./kg. i.v.; and (5) tranylcypromine sulphate 40 mg./kg. i.v.

The MAO inhibitors, phenalzine sulphate and isocarboxazide were given 3 hr prior to arecoline administration while iproniazid and nialamide were given half an hour before. Arecoline given was the subconvulsive dose of 1 mg./kg. i.v.

The effect of tranylcypromine was also studied by giving the drug 24 hr earlier and injecting arecoline the next day.

RESULTS

Amongst the MAO inhibitors phenalzine sulphate slightly enhanced the general activity of the mice with the exhibition of Straub phenomenon. The arecoline tremors after phenalzine appeared to be slightly more intense. Besides the tremors, in a few animals convulsive seizures were seen with the same dose of arecoline.

Isocarboxazide, iproniazid, nialamide and tranylcypromine enhanced the arecoline tremerogenic action.

DISCUSSION

Inhibitors of MAO are also central stimulants. During or shortly after treatment with an MAO inhibitor toxic reactions may follow the use of some other centrally acting drugs like imipramine, pethidine, amphetamine, etc.

That the arecoline tremors are probably due to the stimulation of the central nervous system can be inferred by the fact that these tremors could still be induced by arecoline after blocking the peripheral autonomic nervous system by methyl atropine or probantheline bromide[42]. Also, arecoline by itself is an MAO inhibitor though of a weak potency. The potentiation of its toxicity by MAO inhibitors may possibly be explained by the additive effect of the two compounds. Unlike its potentiation of arecoline tremors, iproniazid has been reported to lessen the onset of tremorine-induced tremors[43] indicating the differences in the mode of action between these compounds in inducing tremors. Tremorine, like arecoline, produces parasympathetic stimulation—salivation, lacrymation, diarrhoea, urination and centrally mediated fast tremors.

Effect of chlorpromazine and benactyzine on arecoline tremors. Chlorpromazine 2 mg. kg. s.c. did not influence the arecoline tremor when the latter was given 2 hr after chlorpromazine sedation. Benactyzine 1 mg. kg. i.v. to mice exhibited no direct effect but completely neutralized the tremor induction by arecoline given 5 min. later. The benactyzine influence lasted for about half an hour.

Atropine sulphate 1 mg. kg. also inhibited the arecoline tremors. But in both the duration of action and extent of antagonism it was less active than benactyzine. The antagonism lasted for about 15 min.

Probantheline bromide and arecoline. Probantheline bromide (Probanthine) 1 mg. kg. i.v. did not antagonize tremor induction by arecoline 1 mg. kg. i.v. in mice. This drug exhibited peripheral antiarecoline action. The complete blockage of *in vitro* arecoline contraction of mouse intestine by 'probanthine' can be seen in Plate II (a).

These studies also indicate that the tremor induction by arecoline is one of CNS stimulation and not peripheral mode of action. Similarly, methyl atropine which cannot pass the blood brain barrier and is hence, only a peripheral parasympathetic blocking agent has been reported to produce marked excitement and tremors in mice after heavy doses of arecoline which normally would have caused death of the animals[42].

The arecoline contractions in rabbits are neutralized by benactyzine and reduced in intensity by atropine and chlorpromazine.

Our results in mice while simulating these in rabbits slightly differ in the influence of chlorpromazine on arecoline contractions. In rabbits chlorpromazine appears to reduce the intensity while no such effect was seen in mice. Whether this is a species difference or the influence of dose-time relationship, needs further elucidation.

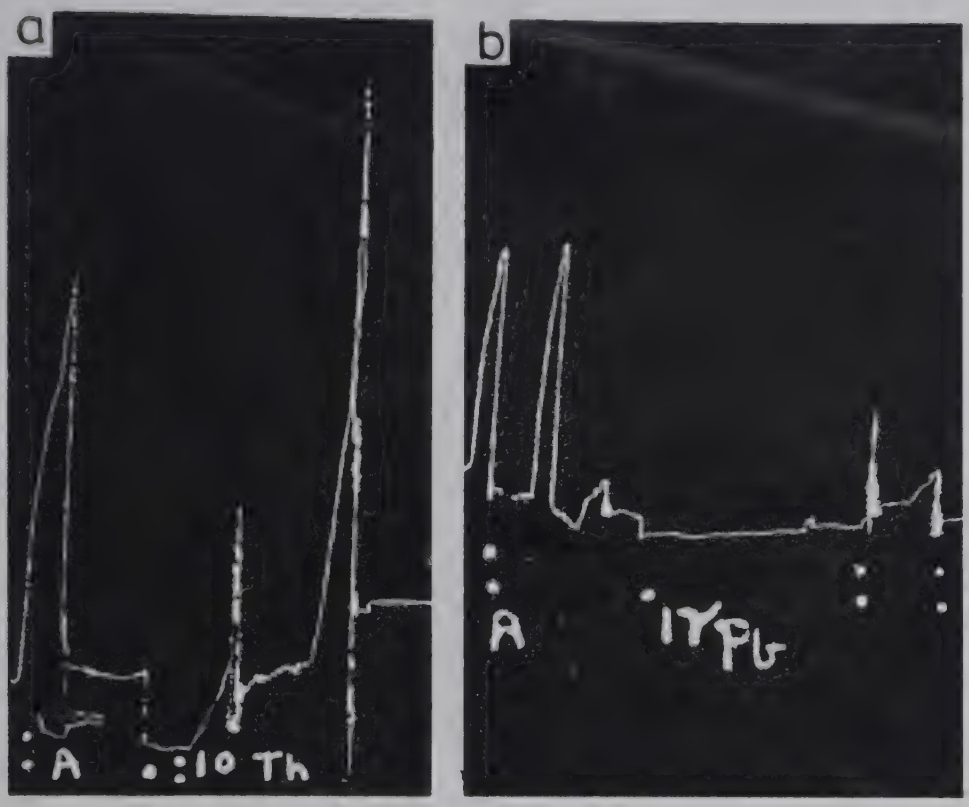

PLATE II—(a) RAT ILEUM (A, Arecoline; Th., thiamine hydrochloride. Note 10γ/ml. of thiamine neutralizes arecoline effect; recovery after wash quick); (b) MOUSE ILEUM (Complete blockage of arecoline effect by probantheline bromide is seen; A, arecoline; Pb, Probantheline bromide, 1γ/ml.)

Tannin and ethyl extracts of areca nut and arecoline tremor. Tannin and ethyl acetate extracts 40 mg./kg. i.v. did not influence arecoline action. Neither potentiation nor antagonist effect was observed on the tremor.

Thiamine and arecoline tremors. Since thiamine antagonizes the contractile effect of arecoline on rat intestines (Pate II b), its influence on CNS stimulation of arecoline was studied.

Initially thiamine hydrochloride was given i.v. at varying doses to male mice. Ten mg./kg. was found to be non-toxic. Mice treated with this dose of thiamine were 10 min. later given arecoline 1 mg./kg. i.v. The duration and extent of tremor was not modified in any way. Thiamine hydrochloride thus appears to influence only the peripheral effects and not CNS stimulation.

Whether this absence of CNS antagonism is due to insufficient concentration of the vitamin at the receptor level in the brain or is a manifestation of a definite qualitative variation remains to be elucidated. A similar peripheral parasympathetic blockage with no influence on central nervous system exhibited by probantheline bromide and methyl atropine is explained as due to the inability of these drugs to cross the blood-brain barrier.

These experimental results are in consonance with the clinical results obtained in schizophrenic patients. Trials of arecoline in such patients

protected by probantheline bromide showed antiechizokinetic action in 1–2 min after injection, reached a peak in 5 min and disappeared completely in 15–20 min. The effect on patients of ipronizid was slightly more prolonged after arecoline. Concomitant therapy with chlorpromazine did not modify or exaggerate either pharmacological or behavioural effects[29].

It is thus seen that arecoline tremors are the results of CNS stimulation by arecoline; MAO inhibitors — both the hydrazine and the non-hydrazines appear to enhance the toxicity of arecoline; the tranquillizer, chlorpromazine does not influence the arecoline effect; atropine and benactyzine inhibit the arecoline tremors while probantheline bromide had no central anti-arecoline action, though it inhibited the peripheral effects, and thiamine which antagonizes the peripheral arecoline action does not influence arecoline tremors.

REFERENCES

1. Charak Samhita, by Agnivesa, Third Edition (1941), Nirvanasagar Press, Bombay.
2. Sushruta Samhita, (1859), C. K. Sen & Co. Ltd, Calcutta.
3. Hareetha Samhita (1849), Sri Venkateshawara Press, Bombay.
4. Rajanighantu (1925), Anandashrama Publications, Poona.
5. Dhanwantari Nighantu, Anandashrama Publications, Poona.
6. Bhavaprakasa, Chowkamba Sanskrit Series, Varanasi.
7. Kaideva Nighantu, Chowkamba Sanskrit Series, Varanasi.
8. Pauly, M., Tobacco Alkaloids and Related Compounds. Proceedings of the Fourth International Symposium, ed. W. S. Von Euler, (1964), p. 15. Pergamon Press.
9. Euler, U. S. Von & Domeij, B., *Acta Pharmacol et. toxicol.*, **1** (1945), 263.
10. Dessinko, Pharmacological Analysis of Central Nervous Action. Proceedings of the First International Meeting, ed. D. M. Paton, Vol. 8, (1962), p. 199. Pergamon Press.
11. Ilyutchenok, R. I., *ibid.*, (1962), 211.
12. Lalitha Kumari, H., Sirsi, M. & Govindarajan, V. S., *Indian J. exp. Biol.*, **3** (1965), 69.
13. Lalitha Kumari, H. & Sirsi, M., Proceedings of the Thirtysecond Mysore State Medical Conference, Mangalore (1965).
14. Nagarajan, G. R. & Seshadri, T. R., *J. sci. industr. Res.*, **20B**, (1961), 615.
15. Govindarajan, V. S. & Matthew, A. G., *Phytochemistry*, **2** (1963), 231.
16. Lalitha Kumari, H. & Sirsi, M. (unpublished observations).
17. Geissmann, T. A., Chemistry of Flavonoid Compounds, Pergamon Press, Oxford (1962).
18. Masquelier, T., The Pharmacology of Plant Phenolics, Academic Press, 1959, p. 113.
19. Das, D. N., *J. Indian chem. Soc.*, **39** (1962), 849.
20. Clark, W. G. & Geissmann, T. A., Biological Antioxidants. Trans III Conf. Josiah Macy Jr Foundation (1948).
21. Lockett, M. F., Pharmacology of Plant Phenolics, Academic Press, (1959), p. 81.
22. Lavollay, J. & Neumann, J., Pharmacology of Plant Phenolics, (1959), p. 103.
23. Ambrose, A. M. & De Eds, F., *J. Pharmac.*, **90** (1947), 359.
24. Salva, J. A., Neumann, J. & Lavollay, J., *C. R. Acad. Sci.*, **227** (1948), 1170.
25. Sirsi, M., Dorle, A. K. & Govindarajan, V. S., *Licentiate*, **13** (1963), 22.
26. Sirsi, M., Dorle, A. K. & Govindarajan, V. S., *Curr. Sci.*, **32** (1963), 455.
27. Herz, A., *Archiv fur Expt. path u. Pharmacol.*, **842** (1962), 414.
28. Votava, Z., Benesova, O., Metysova, J. & Soucskova, M., Psychopharmacological Methods, ed. Votova, State Medical Publishing House, Prague, (1965), p. 84.
29. Zirkle, C. L. & Kaiser, C., Medicinal Chemistry, Vol. 4, ed. Maxwell Gorman (1964), p. 445.

30. Udenfriend, S., Witkop, B., Redfield, B. G. & Weissbach, H., *Biochem. Pharmac.*, **1** (1958), 160.
31. Sjoerderma, A., Smith, T. E., Stevenson, T. D. & Udenfriend, S., *Proc. Soc. Expt. Biol. Med.*, **89** (1955), 36.
32. Udenfriend, S., Weissbach, H. & Clark, C. T., *J. biol. Chem.*, **215** (1955), 339.
33. Biel, J. H., Horita, A. & Drukker, A. E., *Med. Chem.*, Vol. 4, Phychopharmacology agents, **1** (1964), 359.
34. Sarkar, S., Banerjee, R., Ise, M. S. & Zeller, E. A. *Helv. Chim. Acta.*, **43** (1960), 439.
35. Meier, R., Gross, F. & Tripod, J., *Klin Wochenschr.*, **32** (1954), 445.
36. Mattila, M. J., Liisa, A. & Vartiainen, A., Tobacco Alkaloids and Related Compounds. ed. Von Euler, Pergamon Press, (1965), 321.
37. Ochs, S., Elements of Neurophysiology, John Wiley & Sons, New York, (1965), p. 440.
38. Ochs, S., *ibid.*, (1965), p. 441.
39. Feldberg, W. Pharmacological Analysis of Central Nervous Action, Proc. First International Meeting, Pergamon Press,(1962), p. 231.
40. Day, C. A. & Yen, H. C. Y., *Fed. Proc.*, **21** (1962), 323.
41. Larsen, P. S. & Silvetti, H., Tobacco Alkaloids and Related Compounds, Pergamon Press, (1964), p. 105.
42. Pfeiffer, C. F. & Jenny, E. H., *Ann. N. Y. Acad. Sci.*, **66** (1957),753.
43. Stern, P. & Gasporovik, I., Pharmacological Analysis of Central Nervous Action. Proc. First International Meeting, Pergamon Press, (1962), p. 149.

Some Recent Advances in the Chemistry of Tropanes

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According to Barton the structure of a natural product is known if (a) its constitution, (b) its configuration and (c) its conformation are elucidated. The understanding of the biogenesis is the next involvement and also the correlation of structure with pharmacological activity.

The tropane alkaloids offered many challenges in all those aspects. Their constitution was elucidated by Willstatter and his followers, Robinson, and Stoll. Also Barger and Schopf contributed considerably to this field.

The knowledge of the relative configuration of the main tropane bases, e.g. atropine, cocaine, scopolamine resulted mainly from stereospecific reactions in the Fifties. Total syntheses helped to confirm those conclusions as reviewed[1]. As to the absolute configurations, cocaine[1,2] was correlated with (−) glutamic acid by Hardegger, while (−) tropic acid, present in hyoscine and hyoscyamine, was proved to have the S-configuration by one of us with (the late) Gy. Csepreghy.

The formation of the tropane alkaloids in the plant is now well understood; labeled ornithine was incorporated into hyoscyamine and this 'primary alkaloid' became oxidized enzymatically to hyoscine[2] subsequently.

Stereochemical investigations were useful in ascribing[3] certain pharmacodynamic actions to a given geometry. For instance, the 3α-tropanyl esters hyoscyamine and hyoscine have a mydriatic, i.e. parasympathetic blocking effect while 3 β-tropanols, cocaine and tropacocaine and psicaine cause anaesthesia. Quaternary tropanium salts, however, have little or no stimulating effect on the central nervous system but they maintain parasympathetic blocking qualities. Alkylidene (or aralkylidene) bistropanium salts, on the other hand, have a curare-like effect provided the interpros-

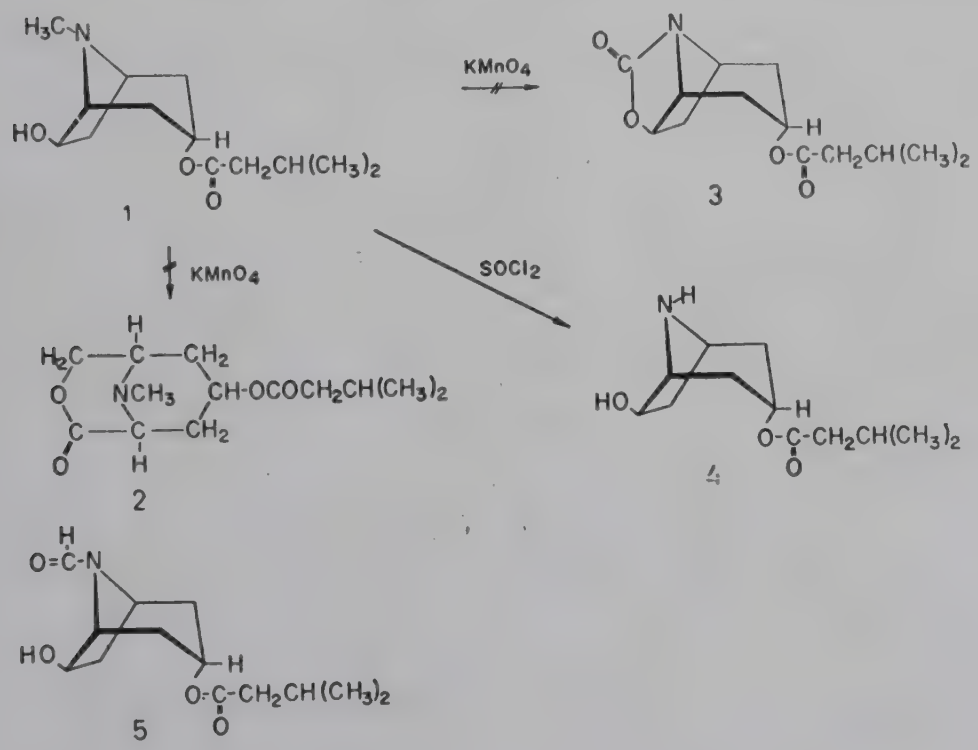
thetic distance of 13–15°A between two cationic centres as postulated by Pfeiffer and his school is being maintained.

Three years ago, the main structural and stereochemical problems had already been solved. However, a practical synthesis of hyoscine seemed desirable. Furthermore, Djerassi called the attention of the speaker to a new point of view: the mass spectrographic investigation of the tropanes. The absolute configuration of some tropane alkaloids was not settled with accuracy.

The biogenesis of this class of alkaloids posed also some interesting questions. Let me turn now to these most recent development in this field.

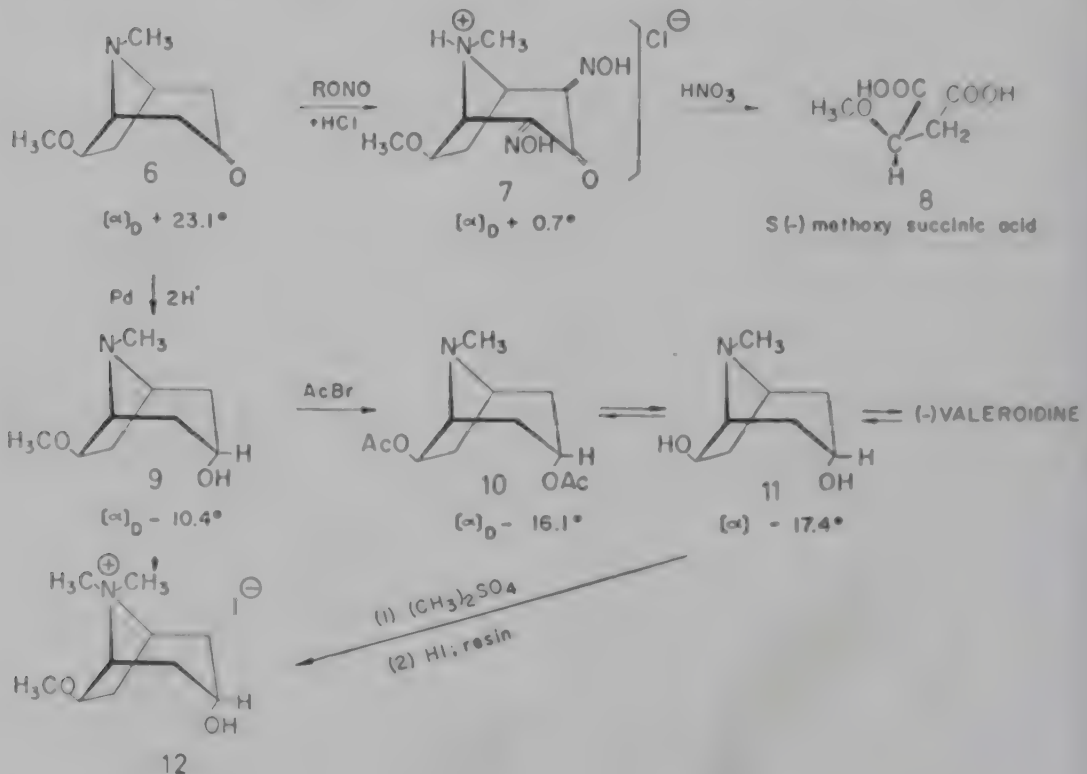
(a) First, an early observation made by Mitchell *et al.*[4] concerning the constitution of a product (m.p. 134°) obtained by oxidation from valeroidine (1) with permanganate was revised. The original of a lactone (2) was replaced soon by a cyclic urethane (3), based on analytical figures. This fact was considered by us formerly[1] as the first evidence in favour of the *cis* position of the C_6-hydroxyl in valeroidine. We were unable to get this urethane from norvaleroidine (4). Furthermore, the IR spectra of presumably (3) showed a strong hydroxyl at 3200 cm.$^{-1}$ and in the NMR it has a proton signal at δ 8·05, an evidence for a formyl proton (in *N*, *N*-dimethylformamide the formyl proton signal is at δ 8·03). Thus *N*-formyl norvaleroidine (5) depicts the consideration better than (3). This does not however invalidate the relative configuration of that hydroxyl as being since proved by lactone salt formation independently[1].

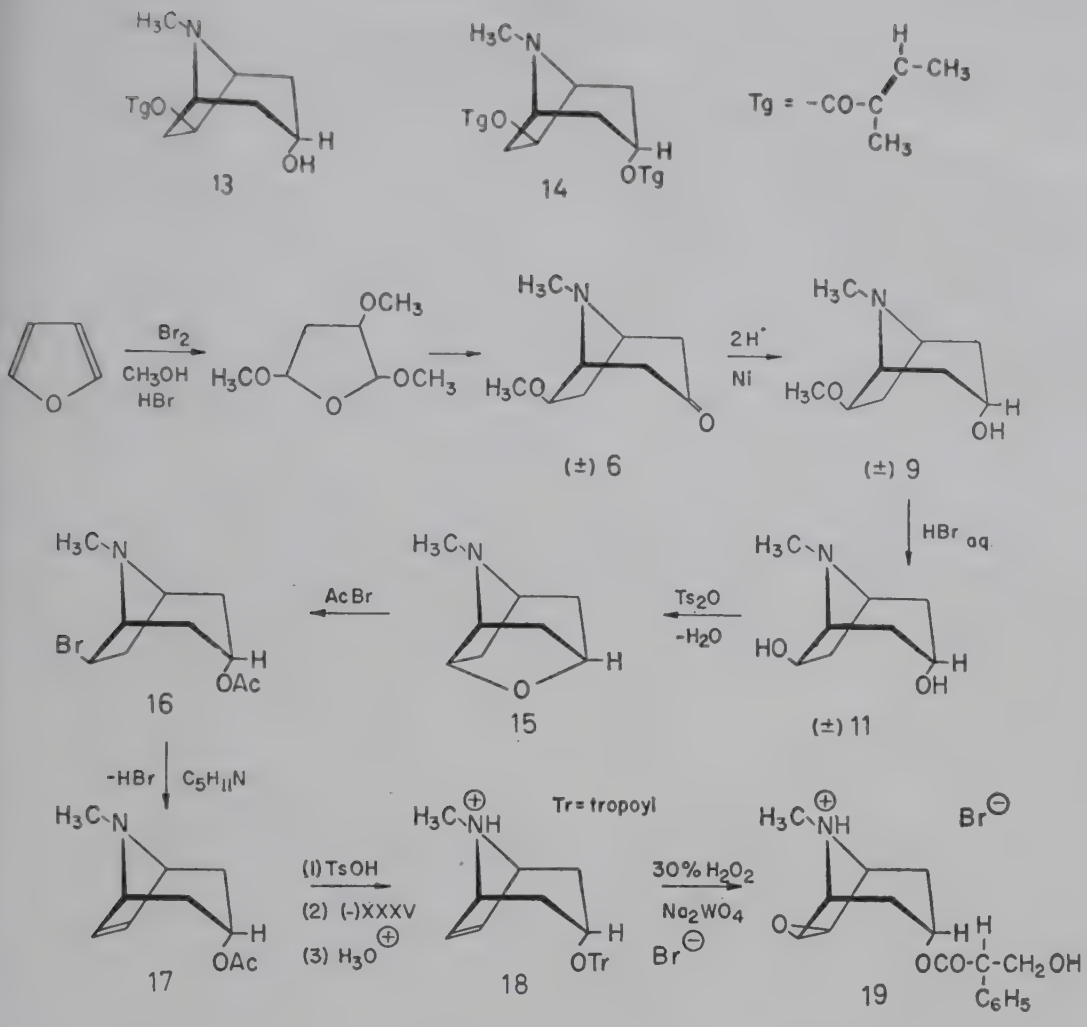
(b) The absolute configuration of valeroidine (1) was correlated recently



by two of us (Fodor and Soti) with that of S(−) methoxysuccinic acid. 6-Methoxytropane-3-one was resolved by (+) tartaric acid and the dextrorotatory derivative (6) converted into the dioximino ketone hydrochloride (7). This was, in turn, oxidized by nitric acid to give S(−) methoxysuccinic acid (8) identical with a specimen obtained from S(−) malic acid by methylation. The dextrorotatory ketone (6) was hydrogenated on the other hand, to 6(−) methoxytropine (9). Correlation of the same with (−) tropane-3α, 6β-diol (11) the alkamine of valeroidine, was achieved in two steps: i) it was subjected to acetobromolysis to methyl bromide and (−) 3α, 6β-diacetoxy-tropane (10) without affecting the C-O at the asymmetric centre of C_6; ii) the (−) 3,6-diacetoxy-tropane proved to be identical in every respect with the product we obtained by acetylating the alkamine of natural valeroidine (1). Subsequently, deacetylation of this diacetate gave pure (−) 3α, 6-β-tropane-diol (10). Finally, (−) 3α, 6β-tropane-diol was converted by selective methylation into 6β-methoxy-tropine methiodine (12).

This provides unequivocal evidence that the relative configuration of the methoxy group in (+) 6-methoxytropane-3-one is β, and the absolute configuration is *S*. Consequently, (−) tropane 3,6-diol from Javanese Coca leaves and also valeroidine have the *S* configuration at C_6. Hence all these compounds possess 3*S*: 6*S* configuration, quite opposite to those deduced in the previous investigation by adopting Hudson's lactone rule[2]. Thus, the





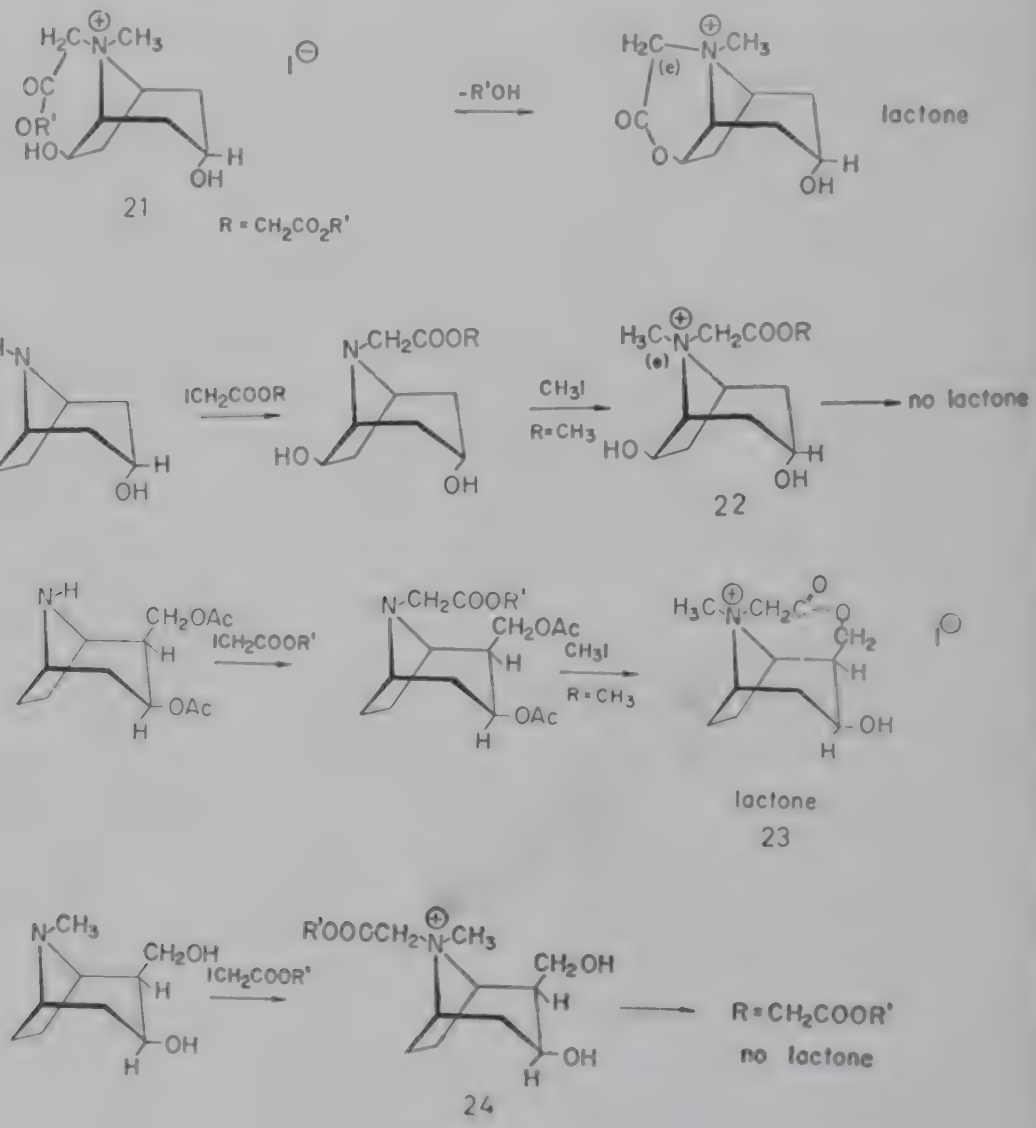
3*R*: 6*R* configuration is valid for the dextrorotatory alkamine both of 6β-tigloyloxytropane-3α-ol (13) from leaves of *Datura cornigera* and of 3α, 6β-ditigloyloxytropane (14) from *Datura ferox*, *innoxia* and *stramonium*.

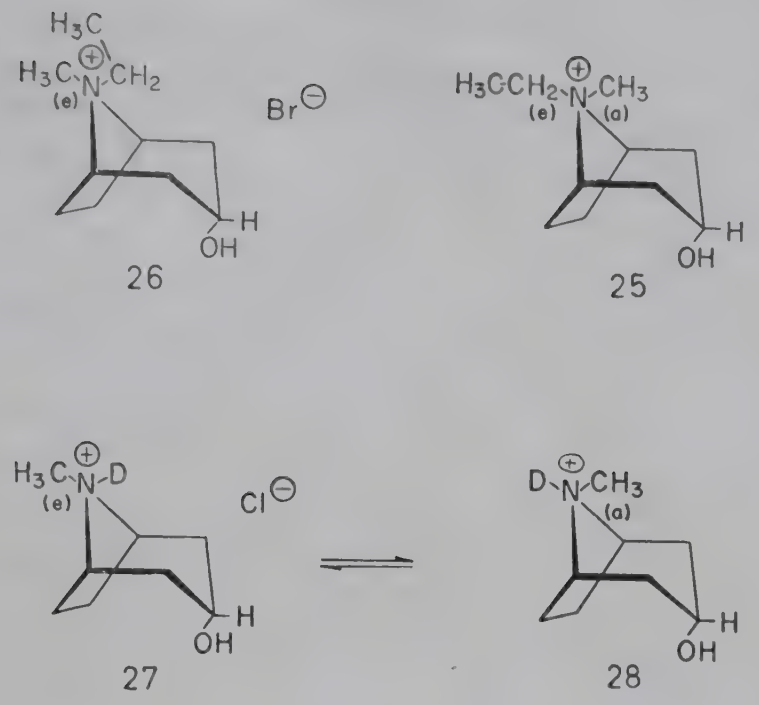
(c) As a further achievement, a 9-step total synthesis of *S*(−) hyoscine was worked out starting with furan, via 6→9→11→15→16→17→18→19. (Kiss, Rakoczi and Fodor).

Some details may be pointed out as new methods. Ether formation (11→15) was achieved by the action of *p*-toluenesulphonic anhydride. Then quantitative dehydrobromination of 6β-bromo-3α-acetoxytropane (16) to 6-tropen-3-yl acetate (17) piperidine was used instead of collidine (yield 10%). And finally, the stereospecific epoxidation of 6-tropenyl-3*S*(−) tropoylate hydrobromide (18) into hyoscine hydrobromide (19) was carried out with 30% aqueous hydrogen peroxide, instead of trifluoroperacetic acid in acetonitrile.

(d) Koczka and one of us (Fodor) found ten years ago a marked steric selectivity in the quaternization of tertiary amines with a tropane skeleton. When two different alkyl or carbethoxymethyl groups were successively

introduced into the ring nitrogen one of the possible *N*-epimers was formed exclusively or predominantly, the configuration of which, e.g. (21) or (22), (23) or (24), depended on the sequence in which the individual alkyl or alkoxycarbonyl-alkyl groups were introduced[1-3]. The exact position of the carboxymethyl groups could be determined since these underwent lactone formation when an adjacent hydroxyl group was available. On this basis, the absolute configuration of a number of tropanium salts became elucidated[1-3]. The simple and reliable chemical method of ring closure could not be applied, however, to tropine ethobromide (25) and *N*-ethyl nortropine methobromide (26) which were formed selectively, depending on the sequence of reactions leading to them. MacGillavry at the University of Amsterdam showed that the ethyl group which entered first assumes the axial position while the methyl group which was added in the last step has the equatorial position in case of *N*-ethyl nortropine methobromide (26).

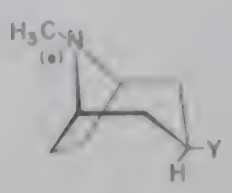




The same regularity was found also in different classes of quaternary ammonium salts, i.e. with those derived from tetrahydroisoquinoline and from morphine and codeine, leading to the formulation of an empirical rule[2].

An explanation for this rather surprising fact was given first by us in terms of a Pitzer strain being present in the five-membered ring of tropanes which should cause the shift of the alkyl group to the axial position in the tertiary amine base. As a consequence, the equatorial position would be available for the quaternizing cation. This interpretation is, however, at variance with recent NMR studies by Closs[5] on tropane deuteriohalides (27, 28) where he found two signals for the *N*-methyl protons. Based upon an analogy with tropine methiodide, he ascribed the signal at lower frequency to the equatorial methyl, and this proved to be the stronger one. Accordingly, he suggested the equatorial position (27) also to be preponderant at the tertiary amine stage. There he prefers the kinetic control to a thermodynamic one as a reason for this selectivity in quaternization reaction. It is hard, however, to accept full parallelism between tertiary amines and their deuterohalides. Accordingly, with the collaboration of Prof. Katritzky (University of East Anglia) we started a more extensive NMR study of tertiary amines of the tropanes series, deuterated in different positions, e.g. in the methyl group, at carbons Nos. 6 and 7 or, alternatively at 2 and 4 or at carbon 3- and of the two epimeric 3-cyanotropanes[6].

Furthermore, X-ray determination of the configuration and conformation of pseudotropine was successful in the last few months. The ring is in the chair form and the methyl on the nitrogen is equatorial, with very strong inter-molecular hydrogen bonding between pseudotropine hydroxyl



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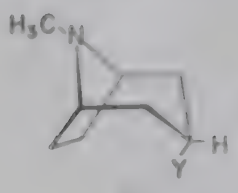

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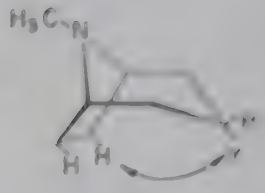

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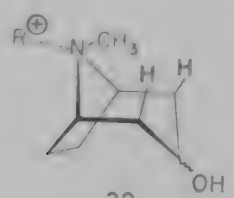
Y = OH; OAc; CN



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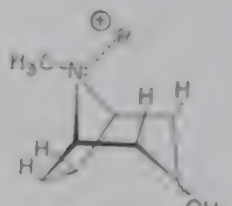


33

and the lone electron pair of another molecule[7]. In addition, dipole moment measurements have been taken by Bishop and us with 3*α*- and 3*β*-cyanotropanes (29) and (30).

The spin-spin splitting of the 3-proton is most informative. Thus the 3 proton in 3 *β*-cyanotropane (29) is a triplet spaced at 9 c. sec., with further splitting at $J = 1{\cdot}5$ c. sec. This corresponds to the chair conformation (29). The dipole moment of (29) was $4{\cdot}04 \pm 0{\cdot}02$ D. This is very close to the calculated value 4·19 D for *N*-methyl and 3*β*-cyano groups both equatorial, i.e. for the classical chair form.

However, the dipole moment found for 3*α*-cyanotropane (30) (3·38 D) is far from that calculated for a normal chair conformation (2·85 D) as well as from that of the boat form (4·17 D). A hypothetical potential energy curve taking into consideration the repulsion between the 6- and 7*α*-hydrogens and the 3*α*-placed cyano group shows a minimum for the total energy at $\Psi = 27{\cdot}5$–$35°$. The calculated dipole moment for that angle is 2·98 D; therefore 67–72 per cent of distorted chair form should be present together with some boat form. The triplet $J = 5$c. sec. found for the *β*-proton is 3*α*-substituted tropanes (Y = CN, or OH or OAc) corresponds to a dihedral angle of 40°. This is in harmony with the assumption based on dipole moments, pointing to a population of conformer (31) in case of 3*α*-tropanols and of 3*α*-cyanotropane.

There is an apparent contradiction between the facts that the equatorial position is more frequently occupied on the tertiary nitrogen in tropane bases and still, the quaternizing cation enters from the equatorial side preponderantly or exclusively.

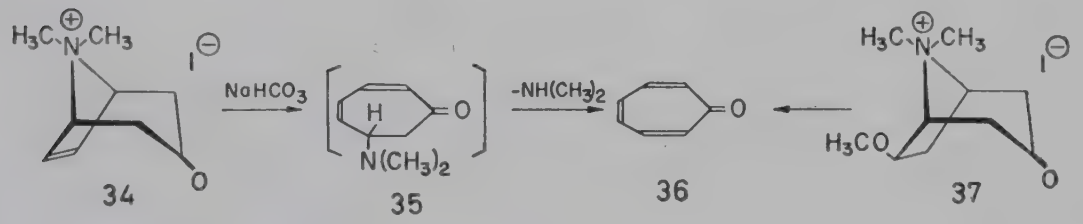
If one considers, however, the steric compression caused by the two axial protons at C_2 and C_4, this makes the equatorial approach, as with (32) more probable than the axial one corresponding to (33). All known facts may be reconciled with that concept.

c) The fragmentation of the tropanes: Tropinone methiodide was subjected to the action of sodium carbonate furnishing by surprise, beside dimethylamine, a nitrogen-free fragment. Willstatter believed it was 'dihydrobenzaldehyde'. However, this possibility was discarded later in favour of a mixture of dihydrotropones (Meinwald, 1955). Similarly, 2-bromotropinone methiodide and 6-hydroxytropinone methiodide could be cleaved to tropone itself (van Tamelen *et al.*, 1956) in a poor yield. 6-Tropen-3-one was prepared from 6-tropen-3α-ol (Fodor, Kiss and Heusner, 1963), by chromic oxide in pyridine; its methiodide (34) gave a quantitative yield of tropone (36) at *p*H 10 within a few minutes (Fodor, 1963). Polarographic analysis of this splitting at *p*H 8 allowed to detect 5-dimethylamino-cyclohepta-2, 4-dienone (35) as an intermediate (Zuman and Horak, 1963), which loses slowly — by raising the *p*H to 11 quickly — dimethylamine to give tropone (36). The same fragmentation occurred with 6-methoxy-tropanone methiodide (37).

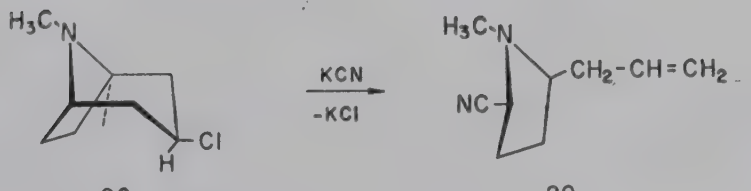
It is curious that cleavage of tropanones occurs but at the quaternary ammonium stage of ring nitrogen, i.e. when it is positively charged.

A more profound fragmentation of the tropanes takes place when potassium cyanide is acted upon 3-chloro-tropane (38) with the formation of *N*-methyl-allyl-5-cyanopyrrolidine (39) (Archer, Lewis and Zenitz, 1957). The epimeric 3-chloro-tropanol gives, on the contrary, 3-cyanotropane owing to transannular archimeric assistance of the basic nitrogen (c.f. Bottini, Grob and Schumacher, 1958). We are now going to trap the presumed intermediate azetidinium salt.

6α-Acetoxy-3β-bromotropane (40), [a minor product besides 3α-acetoxy-6β-bromotropane (16), in the acetobromolysis of 3, 6-oxidotropane (15)] underwent a spontaneous fragmentation to 1, 2-dimethyl-5-vinyl-pyrrole (41).



34 35 36 37



38 39

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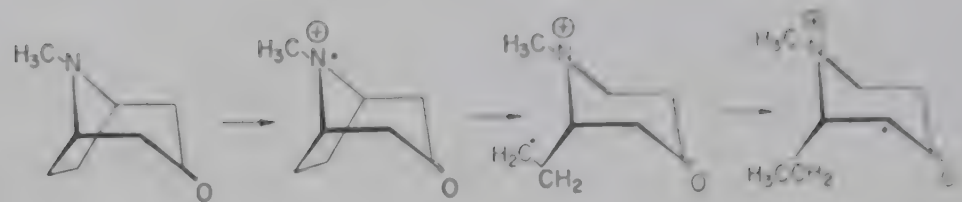
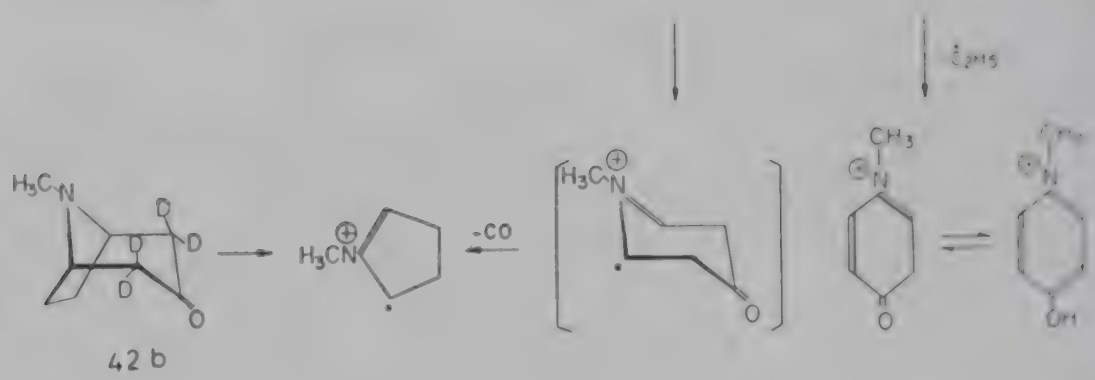


42 a



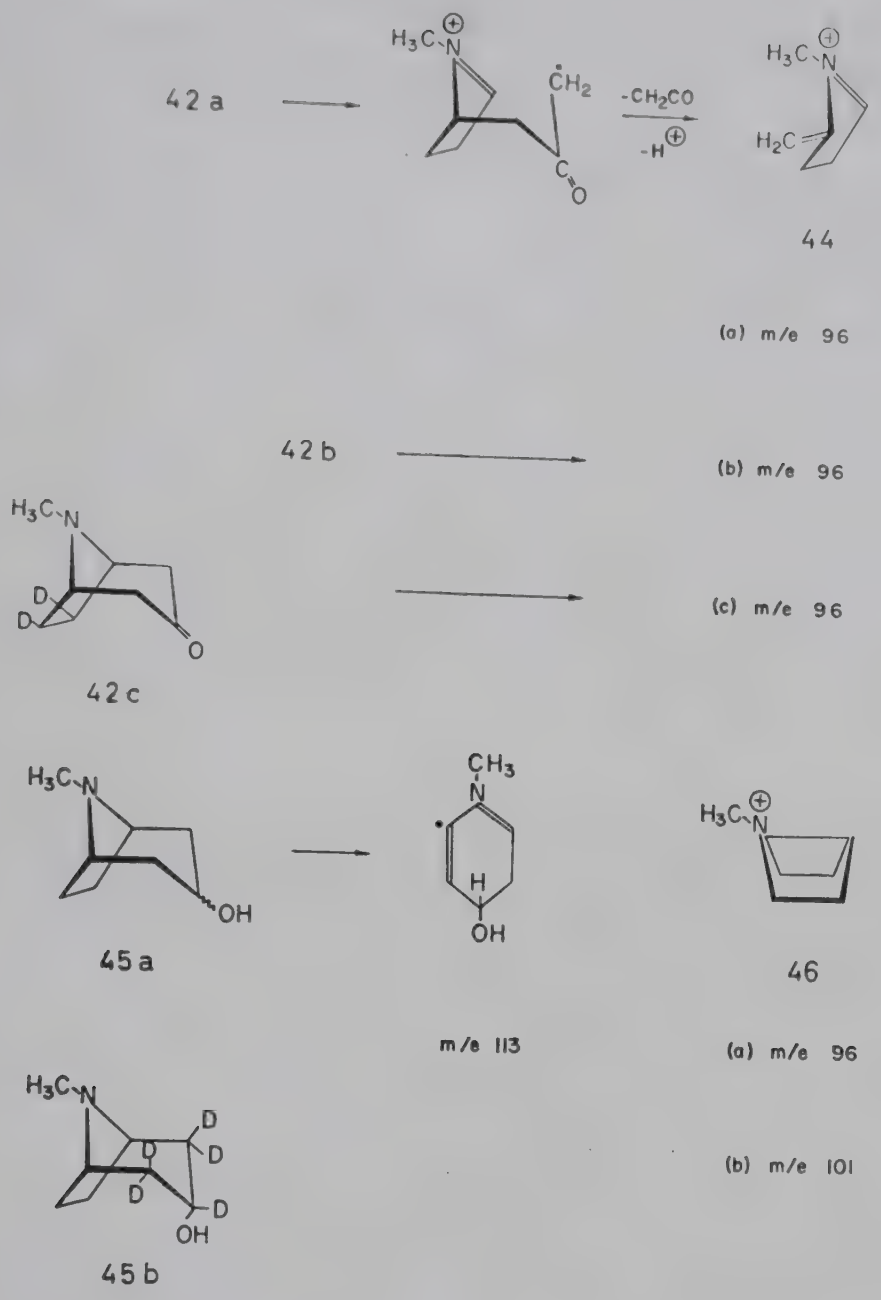
42 b

(a) m/e 83
(b) m/e 87

(a) m/e 110
(b) m/e 114

Very interesting information has been obtained concerning the fragmentation of tropanes under electron impact (Budzikiewicz, Djerassi, Fodor *et al.*, 1964). Investigations started with tropinone (42a) and its 6, 7-dideutero (42c), and with the 2, 2, 4, 4-tetradeutero (42b) derivatives. Shift in the mass of a certain species in the mass spectrum, or no change by going from one series to the other clearly indicates the origin of that fragment in the whole skeleton; e.g. tropinone (42a) and 6, 7-dideutero tropinone (42c) both gave a fragment *m/e* 83 while 2, 2, 4, 4-tetradeuterotropine afforded *m/e* 87.

This is very probably formed from a part of the 6-membered ring: 5, 6 and 1, 7-fission with subsequent loss of CO to give the *N*-methyl-1 (2) pyrroleninium ion (43). An alternate bond breaking, i.e. C_1 C_2 leads with loss of ketene (C_3 C_4 splitting) and of the C_5-H to a fragment *m/e* 96. When starting from any of the deuterated samples, this shifts to *m/e* 98, substantiating the constitution of a 2-methylene-*N*-methyl-1 (5) pyrroleninium ion (44).



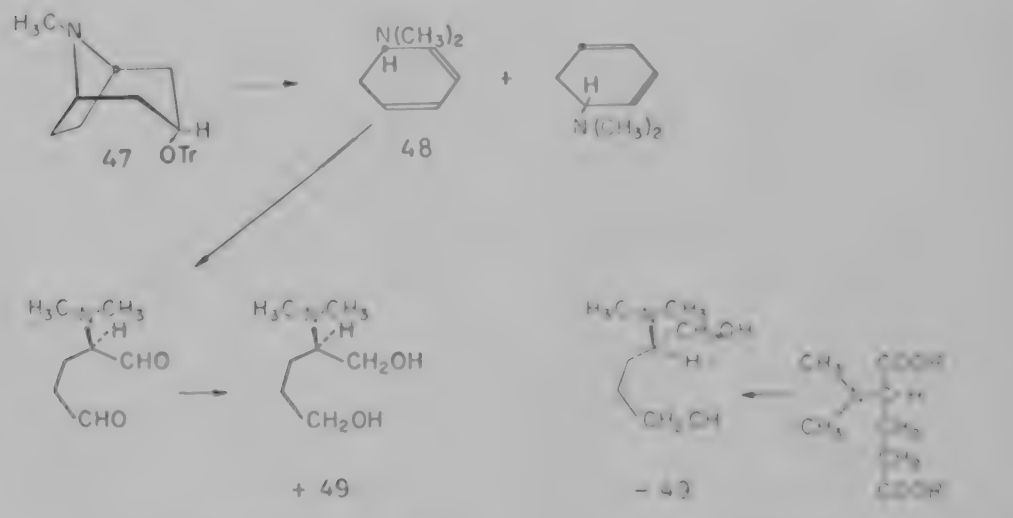
The same technique was adopted in the case of tropan-3-ol (45a) and 6, 7-dideutero tropan-3-ol (45c) where the main species has *m/e* 96; this was shifted, however, in the case of 2, 2, 3, 4, 4-pentadeutero-tropan-3-ol (45b) to *m/e* 101. This shows that all five hydrogens have been retained during impact, rationalized best in terms of a bridged *N*-methyl-(4H)-pyridinium ion (46) (Budzikiewicz *et al.;* cf. Parello *et al.*, 1964).

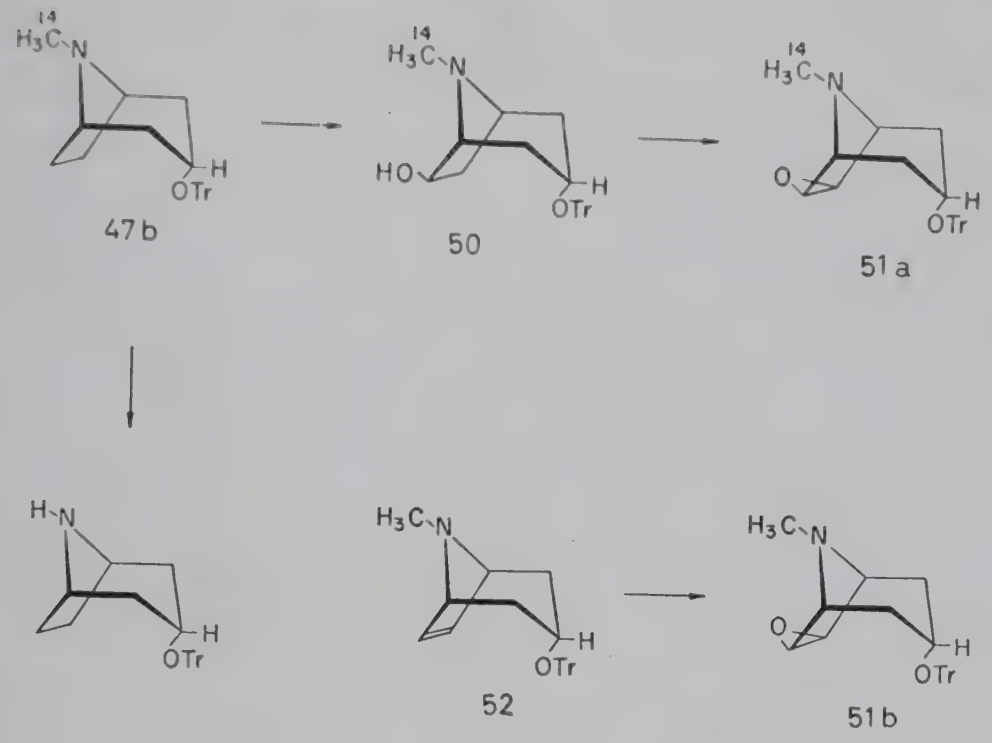
Ecgonines and a lot of other tropanes too have been submitted to mass spectrometric investigation, enabling the chemist to have snap-shut information by taking the mass spectrum of a hitherto unknown alleged tropane base, using a few milligrams of the substance only.

The biogenesis of the tropane alkaloids has been and is still one of the most attractive fields. It was Prof. Leo Marion who first gave a precise answer to the origin of the tropane skeleton based upon tracer work. He succeeded with Draper and Kirkwood as early as 1951 to prove that *Datura stilli* is unable to convert labeled putrescine into hyoscyamine while practically the whole label was recovered by Marion, Leete and Spencer when ornithine-2-^{14}C was used in feeding experiments. The label was detected in the bridgehead of the atropine base and it was assumed that a symmetrical incorporation took place.

Concerning the origin of carbons atoms 2, 3 and 4, Mothes and his coworkers could show in 1961 that they arise from acetoacetyl coenzyme A. Furthermore in 1962, Leete and independently of him, Bothner-By have shown by systematic degradation of hyoscyamine that the incorporation of radiocarbon No. 2 of ornithine took place in only one position, either the 1 or the 5 of hyoscyamine (47a). Since Leete correlated the configuration of the dimethylaminocycloheptadiene (48), containing the whole label after resolution, with D-dimethyl glutaminol (49) we now know the exact position of the label in the tropane skeleton: it is on C-1.

In view of these recent developments it is surprising that Mothes *et al.* (1963) recorded the use of 1, 4-labeled putrescine in feeding sterilized root cultures of *Datura*, leading to the formation of radioactive (47) with high content of radiocarbon at C_1 and C_3 as proved by degradation to radioactive *N*-methyl succinimide. That proves the possibility of a second alternative pathway leading to hyoscyamine in the plant tissue. This is surprising since Romeike and Fodor failed in 1962 to find any incorporation of 2, 3-^{14}C-labeled succinic dialdehyde into the tropane skeleton. According to Mothes, ornithine is not converted into putrescine; they follow quite different routes in biogenesis.





In our opinion, however, ornithine has to be the main precursor while putrescine might be carboxylated to it before being incorporated into the tropane skeleton. This question could be answered quickly if the degradation of the product from putrescine and resolution of the 'tropidine' were carried out following the lines of Leete.

Romeike and Fodor could show in a systematic research work that hyoscyamine (47b) is converted by the so-called alkaloid-free plant into hyoscine (51), the intermediate being 6-hydroxy-hyoscyamine (50). Dehydro-hyoscyamine is also oxidized to (51). These investigations have been completed by the tracer method in very young *Datura* plants. However, nothing is known about the role of 6, 7-dihydroxylated tropane bases in metabolism. Teloidine occurs so far only as tiglic acid ester but not as tropic acid ester. Of course nobody knew the role of 6-hydroxyhyoscyamine before, when tropane-3, 6-diol was available only as its isovaleric ester. Accordingly it was deemed of interest to synthesize tropoyl-teloidine for feeding experiments. This work is now in progress.

(g) The stereoselective quaternization of *N*-epimeric tropanols enabled the synthesis and pharmacological investigation thereof (Doda *et al.*, 1963). The strongest ganglion blocking activity was found with an axial ethyl and an equatorial *p*-phenyl benzyl tropanium salt. The *N*-epimer is much weaker.

As you may see, the tropane field was enriched even in the last years by some newer methods and findings.

REFERENCES

1. Fodor, G., The Alkaloids, Vol. 6, (1960), p. 145, Academic Press, New York.
2. Fodor, G., *Chem. & Ind.*, (1961), 1500.
3. Nador, K., Recent Developments in the Chemistry of Natural Carbon Compounds, Vol. 1, (1965), p. 163. Akademiai Kiado, Budapest.
4. Mitchell Wm. F. & Martin, W. F., *J. chem. Soc.*, (1949), 1173; Mitchell, Wm. F. & Trautner, E. M., *J. chem. Soc.*, (1947), 1330.
5. Closs, G. L., *J. Am. chem. Soc.*, **81** (1959), 5456.
6. Bishop, R. J., Fodor, G., Katritzky, A. R., Soti, F., Sutton, L. E. & Swarbrick, F. J., *J. chem. Soc.*, **C74** (1966).
7. MacGillavry, C. H. (unpublished).
8. Doda, M., Gyorgy, L. & Nador, K., *Arch. int. Pharmacodyn.*, **145** (1963), 264.

N-β-Phenylpropionamides Derived From 1,2-Diphenylethylamines as CNS Depressants

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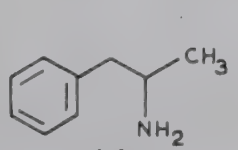
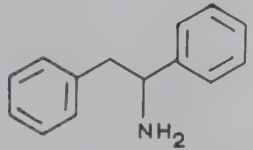
Introduction

We have been interested in the last few years in the synthesis of arylalkylamines and amides in order to study their physiological activity and if possible to arrive at structure-activity relationship in this field. Whereas derivatives of benzylamine and phenylethylamine have been extensively studied by previous workers, only a limited amount of work has been done in the field of diphenylethylamines. Some of these have been studied for antimitotic[1] and antitumor[2] activity for the reason that 1, 2-diphenylethylamine contributes to the partial structure of narcotine and chelidonine.

1, 2-Diphenylethylamine was first studied for its action on CNS by Gunn and Gurd[3] in 1940 when they compared it to benzedrine (I) and noted that replacement of the methyl group in benzedrine with a phenyl group to form 1, 2-diphenylethylamine (II) changes the stimulant action of benzedrine to a depressant action.

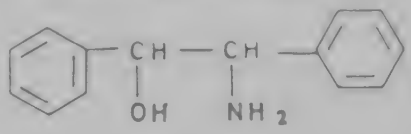


(I)
Benzedrine



(II)
1,2- Diphenylethylamine

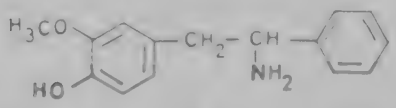
N-Alkyl derivatives were studied by Tainter and collaborators[7] and Goodson and collaborators[8]. The most interesting paper in the series was from Dodds[9] who first reported the synthesis of various 1, 2-diphenylethylamines in 1943, tested them for morphine-like action and found them to exhibit analgesic activity in varying degrees. The compounds containing methoxyl and hydroxyl groups had the capacity to depress the righting reflex in rats, to raise blood sugar in rabbits and to cause hyperexcitability in cats. Among the compounds which showed definite activity, (II) and (III) were recommended for clinical evaluation. Compound (III) was found to be most active in doses of 200 to 400 mg, given orally at four-hour intervals to cancer patients with intractable pain, where it could replace morphine[7]. Clinical evaluation of (III) in fourteen patients showed complete relief from pain associated with nerve pressure without undesirable side effects, but it caused the subjective symptoms of mild drunkenness[8]. None of the compounds however found use as therapeutic agents.



(III)

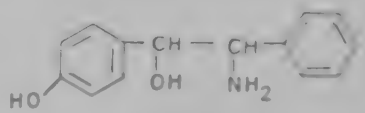
2-Hydroxy-1,2-diphenylethylamine

McPhee and Erickson Jr.[9] following a parallel line of approach synthesized 1, 2-diphenylethyl and 1, 2-diphenylethanol amines to test them for analgesic activity. They observed that several of the compounds had a definite analgesic action. The most effective among these compounds were the hydrochlorides of (IV) and (V).



(IV)

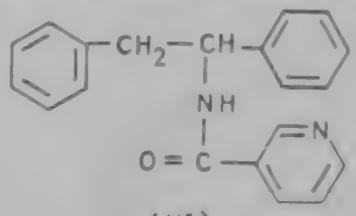
1-Phenyl, 2-(3-methoxy-4-hydroxyphenyl) ethylamine



(V)

1-Phenyl, 2-(4-hydroxyphenyl)-2-hydroxy-ethylamine

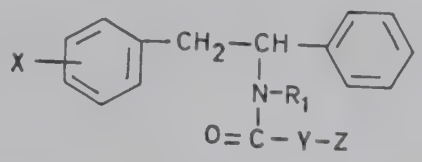
The only diphenylethylamine derivative which has found use in medical practice is *N*-nicotinoylamino-1, 2-diphenylethane[10], which has been marketed since 1948 as an antispasmodic agent by Cilag under the name *Lyspamine* (VI). Martin and Habicht[11] have also studied basic amides



(VI)

N-Nicotinoylamino-1,2-diphenylethane (Lyspamine)

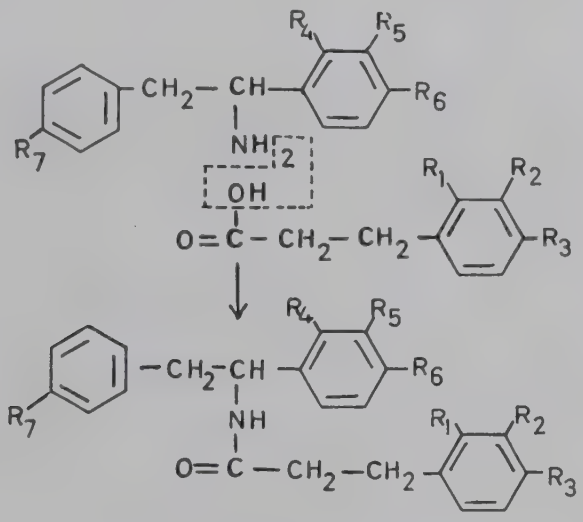
derived from 1, 2-diphenylethylamine of the general structure (VII) for anticonvulsant activity.



(VII)

R_1 = H, alkyl, alkenyl or aralkyl
X = H, hydroxy or alkoxy
Y = alkylene group
Z = NH_2 or substituted amino group

Compounds of this type appeared to be of interest to us for physiological study. Accordingly, fifty compounds of the general structure (VIII) were synthesized by condensing substituted 1, 2-diphenylethylamines with various β-phenylpropionic acids.

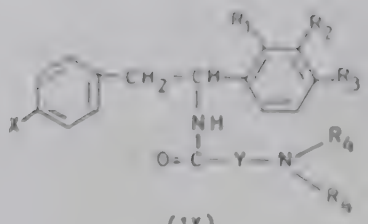


(VIII)

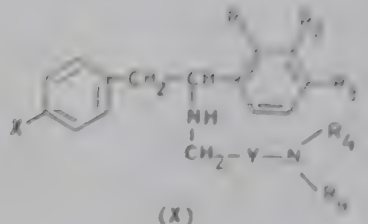
CHEMISTRY

Materials and Methods

1, 2-Diphenylethylamines required for this work were obtained by the reductive amination of the corresponding desoxybenzoins. β-Phenylpropionic acids were obtained as described in literature. Derivatives of 1, 2-diphenylethylamines[12] with different basic side-chains attached to the nitrogen atom were also synthesized to determine differences in the physiological properties of the basic amides (IX) and the corresponding amines (X), and also to compare them with corresponding derivatives of benzylamine and β-phenylethylamine which are also being synthesized by us as a separate paper of this overall project.



(IX)



(X)

R_1, R_2, R_3 = H, CH_3 or C_2H_5

$N\langle^{R_4}_{R_4}$ = morpholine, piperidine, diethylamine or pyrrolidine

X = H or Cl

Y = $-CH_2-$, $-CH_2-CH_2-$ or $-CH-$ (CH_3)

Experimental

N-β-Phenylpropionyl-1, 2-diphenylethylamines were prepared by condensing various β-phenylpropionic acids with 1, 2-diphenylethylamines. The required 1, 2-diphenylethylamines were prepared by the reductive amination of desoxybenzoins, which in turn were obtained by the Friedel-Craft condensation between the appropriate aromatic hydrocarbon and phenylacetyl and *p*-chlorophenylacetyl chlorides. The desoxybenzoins thus obtained are described in Table 1.

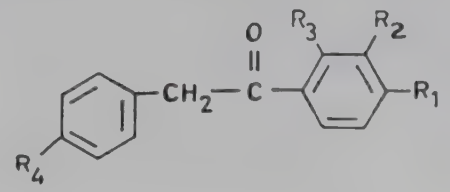
(a) **1, 2-Diphenylethylamines.** Ammonium carbonate (21·5 g. (0·22 mole) was taken in a 3-necked flask fitted with a dropping funnel and a condenser for downward distillation, and formic acid (98%) (20 ml.) was added dropwise. The mixture was heated slowly till the temperature rose to 155° C.; the desoxybenzoin (0·1 mole) was then added in one lot. Heating was resumed and the temperature maintained at 180–85° C. for 8 hr. The reaction mixture was then heated with 100 ml. of 1 : 1 hydrochloric acid for 6 hr, cooled and basified with 20 per cent aqueous sodium hydroxide, extracted with ether, dried over anhydrous sodium sulphate and the product isolated by distillation under reduced pressure. The amines thus obtained are described in Table 2.

(b) **β-Phenylpropionic acids.** β-Phenylpropionic acid was supplied by Aldrich & Co. Inc., New York. 2-[20], 3-[20] and 4-chloro[21]; 2-[22], 3-[23] and 4-methoxy[24]; 4-methyl[25]; 3 : 4-methylenedioxy[26]; 3 : 4-dimethoxy[27], 3-methoxy-4-ethoxy[28]; 3 : 4-diethoxy[29] and 3 : 4-dichloro[30] phenyl propionic acids were obtained by literature methods. 2-Methyl-3-chloro-β-phenylpropionic acid was obtained as described by Zaheer, Sidhu and Sattur (private communication).

(c) **Condensation of 1, 2-diphenylethylamines with substituted β-phenylpropionic acids.** The appropriate 1, 2-diphenylethylamine

TABLE 1

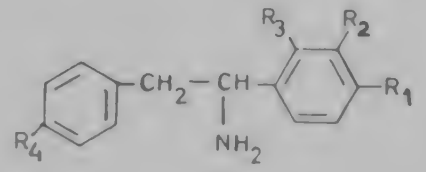
DESOXYBENZOINS



S. No.	R_1	R_2	R_3	R_4	M.P., °C. or B.P., °C./mm.	Mol. formula	Analysis, % Calc. C	Calc. H	Found C	Found H	Reference
1	H	H	H	H	..	..	..	..	..	..	Allen and Barker[13]
2	CH_3	H	H	H	..	..	..	..	..	..	Strassman[14]
3	C_2H_5	H	H	H	..	..	..	..	..	..	Farbenind[15]
4	H	H	H	Cl	..	..	..	..	..	..	Hauser and Morris[16]
5	CH_3	CH_3	H	H	96	$C_{16}H_{16}O$	85·68	7·19	85·88	7·11	
6	CH_3	H	CH_3	H	160-62/1·2	$C_{16}H_{16}O$	85·68	7·19	85·57	7·14	

TABLE 2

1 2-DIPHENYLETHYLAMINES



S. No.	Code No. RRL	R_1	R_2	R_3	R_4	B.P. °C./mm.	Mol. formula	Analysis.[a] Calc. C	Calc. H	Calc. N	Found C	Found H	Found N	References
1	1001	H	H	H	H	..	..	..	..	.				Baccanger[illegible] Peterson[b]
2	1002	CH_3	H	H	H	..	..	..	..	..				Ogata et al.[c]
3	1003	C_2H_5	H	H	H	..	..	..	..	.				Rao[d]
4	1004	H	H	H	Cl	..	..	..	..	.				Rao[d]
5	1005	CH_3	CH_3	H	H	205-07/2	$C_{16}H_{19}N$	85.28	8.50	6.21	85.30	8.79	6.18	
6	1006	CH_3	H	CH_3	H	174-76/2	$C_{16}H_{19}N$	85.28	8.50	6.22	85.09	8.63	6.17	

(0·1 mole) and β-phenylpropionic acid (0·1 mole) were mixed and heated in an oil bath at 180–85 C. for 2 hr. The mixture was then poured into a solution of aqueous sodium bicarbonate (10%; 100 ml.) to remove the unreacted acid. It was filtered and titrated with 1 : 1 hydrochloric acid to remove the unreacted amine, filtered again, dried and recrystallized from dilute ethanol. The compounds thus obtained are described in Table 3.

PHARMACOLOGY

Primary screening for possible CNS activity of some of these compounds was carried out at the Riker Laboratories, North Ridge, Calif., USA, and later at the Pharmacology Research Unit of CSIR at the Department of Pharmacology, Seth G.S. Medical College Bombay.

Gross Behaviour

Primary testing. The primary CNS screening was done in mice. The test compounds were injected intraperitoneally suspended in 0·5% CMC at different concentrations. The parameters used for assessment are shown in Table 4.

All the compounds showed a sedative, tranquillizing activity in mice. LD_{50} by the intraperitoneal route was determined in mice. Out of the 50 compounds screened, the compounds (XI) to (XV) shown in Fig. 1 were selected for further studies.

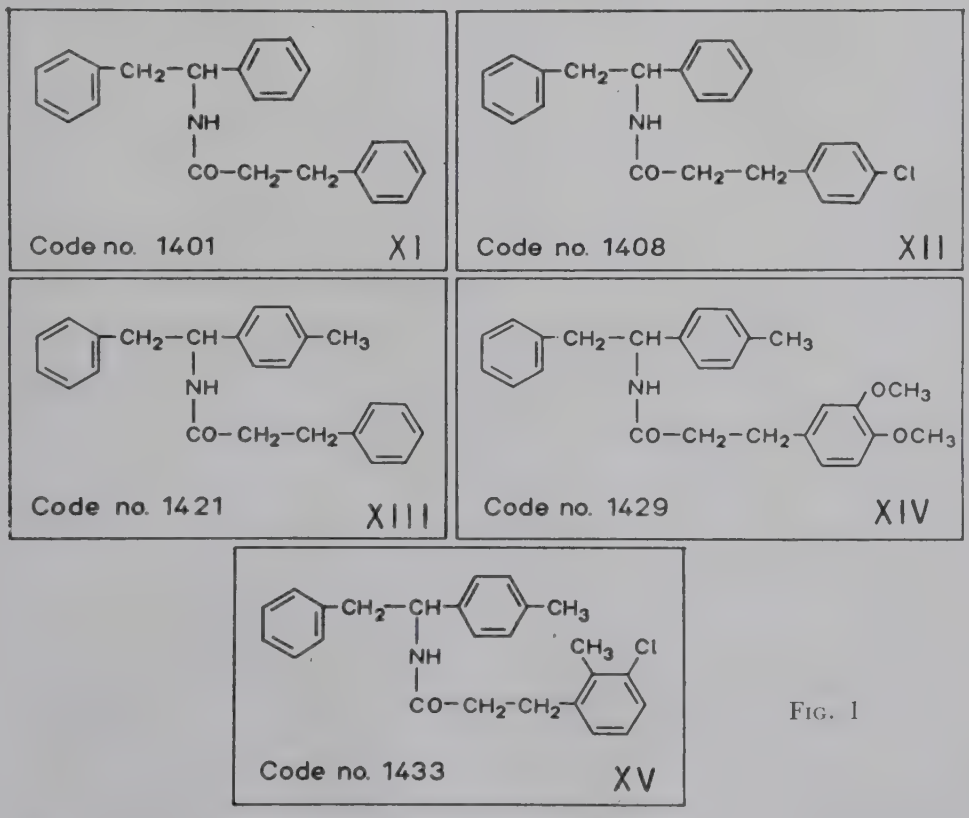
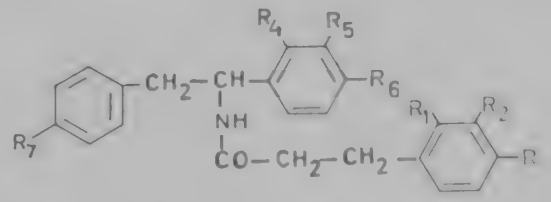


FIG. 1

TABLE 3 N-3-PHENYLPROPIONAMIDES DERIVED FROM 1, 2-DIPHENYLETHYLAMINES



S. No.	Code No. RRL	R_1	R_2	R_3	R_4	R_5	R_6	R_7	M.P. °C.	Mol. formula	Analysis Calc. C	Calc. H	Calc. N	Found C	Found H	Found N
(1)	(2	3)	(4)	(5	(6)	(7	(8)	(9)	(10)	(11)	(12	13	14)	(15	16	17)
1	1401	H	H	H	H	H	H	H	127	$C_{23}H_{23}NO$	83·85	7·04	4·25	83·72	7·48	4·06
2	1402	H	H	CH_3	H	H	H	H	155	$C_{24}H_{25}NO$	83·92	7·34	4·08	83·88	7·09	4·22
3	1403	OCH_3	H	H	H	H	H	H	136	$C_{24}H_{25}NO_2$	80·19	7·01	3·90	80·46	6·92	3·71
4	1404	H	OCH_3	H	H	H	H	H	148	$C_{24}H_{25}NO_2$	80·19	7·01	3·90	80·39	7·17	3·79
5	1405	H	H	OCH_3	H	H	H	H	138	$C_{24}H_{25}NO_2$	80·19	7·01	3·90	80·64	7·14	3·97
6	1406	Cl	H	H	H	H	H	H	132	$C_{23}H_{22}NOCl$	75·93	6·01	3·84	75·70	6·31	3·83
7	1407	H	Cl	H	H	H	H	H	136	$C_{23}H_{22}NOCl$	75·93	6·01	3·84	75·84	6·18	3·73
8	1408	H	H	Cl	H	H	H	H	131	$C_{23}H_{22}NOCl$	75·93	6·01	3·84	76·01	5·90	3·94
9	1409	H	OCH_3	OCH_3	H	H	H	H	141	$C_{25}H_{27}NO_3$	77·09	6·99	3·60	77·20	7·01	3·51
10	1411	H	-O-CH_2-O-		H	H	H	H	129	$C_{24}H_{23}NO_3$	77·19	6·21	3·61	77·43	6·08	3·63
11	1412	H	OC_2H_5	OC_2H	H	H	H	H	122	$C_{27}H_{31}NO_3$	77·66	7·48	3·35	77·48	7·31	3·49

12	1414	H	Cl	Cl	H	H	H	H	124	$C_{23}H_{21}NOCl_2$	64·34	5·28	3·52	64·18	5·46	3·66
13	1421	H	H	H	H	H	CH_3	H	144	$C_{24}H_{25}NO$	83·92	7·34	4·08	84·14	7·25	4·17
14	1422	H	H	CH_3	H	H	CH_3	H	119	$C_{25}H_{27}NO$	83·99	7·61	3·92	83·86	7·79	3·82
15	1423	OCH_3	H	H	H	H	CH_3	H	126	$C_{25}H_{27}NO_2$	80·39	7·29	3·75	80·56	7·42	3·79
16	1424	H	OCH_3	H	H	H	CH_3	H	124	$C_{25}H_{27}NO_2$	80·39	7·29	3·75	80·22	7·15	3·93
17	1426	Cl	H	H	H	H	CH_3	H	134	$C_{24}H_{24}NOCl$	76·30	6·36	3·79	76·08	6·49	3·66
18	1427	H	Cl	H	H	H	CH_3	H	146	$C_{24}H_{24}NOCl$	76·30	6·36	3·79	76·46	6·52	3·87
19	1428	H	H	Cl	H	H	CH_3	H	133	$C_{24}H_{24}NOCl$	76·30	6·36	3·79	76·41	6·25	3·68
20	1429	H	OCH_3	OCH_3	H	H	CH_3	H	118	$C_{26}H_{29}NO_3$	77·39	7·24	3·47	77·17	7·38	3·36
21	1431	H	-O-CH_2-O-		H	H	CH_3	H	122	$C_{25}H_{25}NO_3$	77·49	6·50	3·62	77·76	6·62	3·80
22	1433	CH_3	Cl	H	H	H	CH_3	H	167	$C_{25}H_{26}NOCl$	76·63	6·64	3·58	76·79	6·51	3·43
23	1434	H	Cl	Cl	H	H	CH_3	H	140	$C_{24}H_{23}NOCl_2$	69·90	5·58	3·40	70·07	5·65	3·61
24	1441	H	H	H	H	H	C_2H_5	H	134	$C_{25}H_{27}NO$	83·99	7·61	3·92	84·23	7·76	4·08
25	1442	H	H	CH_3	H	H	C_2H_5	H	132	$C_{26}H_{29}NO$	84·05	7·87	3·77	83·92	7·73	3·88
26	1443	OCH_3	H	H	H	H	C_2H_5	H	133	$C_{26}H_{29}NO_2$	80·58	7·54	3·61	80·74	7·37	3·72
27	1444	H	OCH_3	H	H	H	C_2H_5	H	99	$C_{26}H_{29}NO_2$	80·58	7·54	3·61	80·69	7·68	3·48
28	1445	H	H	OCH_3	H	H	C_2H_5	H	145	$C_{26}H_{29}NO_2$	80·58	7·54	3·61	80·42	7·39	3·76
29	1446	Cl	H	H	H	H	C_2H_5	H	112	$C_{25}H_{26}NOCl$	76·63	6·64	3·58	76·75	6·51	3·67
30	1447	H	Cl	H	H	H	C_2H_5	H	110	$C_{25}H_{26}NOCl$	76·63	6·64	3·58	76·49	6·54	3·71
31	1448	H	H	Cl	H	H	C_2H_5	H	107	$C_{25}H_{26}NOCl$	76·63	6·64	3·58	76·55	6·47	3·64

Contd

TABLE 3—N-β-PHENYLPROPIONAMIDES DERIVED FROM 1, 2-DIPHENYLETHYLAMINES—Cont.

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)	(17)
32	1449	H	OCH_3	OCH_3	H	H	C_2H_5	H	110	$C_{27}H_{31}NO_3$	77·66	7·48	3·35	77·64	7·60	4·01
33	1451	H	-O-CH_2-O-		H	H	C_2H_5	H	113	$C_{26}H_{27}NO_3$	77·78	6·78	3·49	77·92	6·63	3·63
34	1452	H	OC_2H_5	OC_2H_5	H	H	C_2H_5	H	116	$C_{29}H_{35}NO_3$	78·17	7·92	3·14	77·90	8·08	3·61
35	1461	H	H	H	H	CH_3	CH_3	H	112	$C_{25}H_{27}NO$	83·99	7·61	3·92	84·25	7·52	3·80
36	1462	H	H	CH_3	H	CH_3	CH_3	H	114	$C_{26}H_{29}NO$	84·05	7·87	3·77	83·91	7·72	3·95
37	1465	H	H	OCH_3	H	CH_3	CH_3	H	102	$C_{26}H_{29}NO_2$	80·58	7·54	3·62	80·67	7·80	3·70
38	1466	Cl	H	H	H	CH_3	CH_3	H	109	$C_{25}H_{26}NOCl$	76·63	6·64	3·58	76·82	6·78	3·46
39	1467	H	Cl	H	H	CH_3	CH_3	H	99	$C_{25}H_{26}NOCl$	76·63	6·64	3·58	76·77	6·54	3·42
40	1469	H	OCH_3	OCH_3	H	CH_3	CH_3	H	79	$C_{27}H_{31}NO_3$	77·66	7·48	3·35	77·84	7·37	3·11
41	1471	H	-O-CH_2-O-		H	CH_3	CH_3	H	101	$C_{26}H_{27}NO_3$	77·78	6·78	3·49	78·05	6·60	3·66
42	1472	H	OC_2H_5	OC_2H_5	H	CH_3	CH_3	H	106	$C_{29}H_{35}NO_3$	78·17	7·92	3·14	77·91	7·78	4·11
43	1481	H	H	H	CH_3	H	CH_3	H	158	$C_{25}H_{27}NO$	83·99	7·61	3·92	84·24	7·47	4·06
44	1482	H	H	CH_3	CH_3	H	CH_3	H	160	$C_{26}H_{29}NO$	84·05	7·87	3·77	83·87	7·96	3·69
45	1491	H	-O-CH_2-O-		CH_3	H	CH_3	H	132	$C_{26}H_{27}NO_3$	77·78	6·78	3·49	78·01	6·91	3·27
46	1493	CH_3	Cl	H	CH_3	H	CH_3	H	130	$C_{26}H_{28}NOCl$	76·90	6·91	3·41	76·99	6·80	3·31
47	1501	H	H	H	H	H	H	Cl	150	$C_{25}H_{26}NOCl$	75·93	6·05	3·85	76·12	5·93	4·10
48	1503	OCH_3	H	H	H	H	H	Cl	148	$C_{21}H_{24}NO_2Cl$	73·19	6·10	3·56	72·95	6·27	3·66
49	1508	H	H	Cl	H	H	H	Cl	136	$C_{23}H_{21}NOCl_2$	64·34	5·28	3·52	64·26	5·42	3·28
50	1510	H	OCH_3	OC_2H_5	H	H	H	Cl	134	$C_{26}H_{28}NO_2Cl$	71·32	6·40	3·20	71·53	6·58	3·47

TABLE 4—GROSS OBSERVATIONS (MICE)

STIMULANT	AUTONOMIC EFFECTS
Hyperactivity	Labored Respiration
Irritability	Cysnosis
Tremor	Blanching
Convulsions	Reddening
Piloerection	Hypothermia
	Abnormal Secretions
DEPRESSANT	Diarrhoea
	Constipation
Ptosis	Distressed
Sedation	
Anaesthesia	MOTOR ACTIVITY
Ataxia	Increased
Catatonia	
Analgesia	Decreased
Straub's Tail	ANTI-ELECTROSHOCK
Rotating Rod Test	LD_{50} i.p.

The studies are divided into two parts. The first covers pharmacological action and subacute toxicity and the second early clinical trials.

In experimental animals, these compounds were administered mostly intraperitoneally. In human studies the test compound was given orally in soft gelatin capsule.

Behavioral Studies

The behaviour of cats, monkeys and adult mongrel dogs were observed before and after administration of the test compounds. Animals were observed for spontaneous motor activity, gait, posture, behaviour towards observers, response to handling, interest in surroundings etc. Aggressiveness in cats was studied by observing their response to the presence of a mouse in the cage. Observations were recorded for an hour prior to administration of the drug, at hourly intervals, thereafter for the next 6 hr and finally at the end of 24 hr.

Compound 1421 was injected intravenously in cats in doses of 5 and 25 mg./kg. and also intraperitoneally in doses of 50 and 100 mg./kg. Sedation, drowsiness, and decrease in activity was noted 1 hr after administration of the drug. The cat was no longer interested in the mouse, did not approach the observer, and preferred to lie down quietly. After intravenous administration there was a decrease in muscle tone leading to inability to stand up. The muscular relaxation was not so marked on intraperitoneal

administration. The effect lasted for about 5 to 6 hr. and the rats recovered from drug effect after 24 hr. No autonomic actions like salivation, urination were observed. Oral administration at a dose of 20 mg./kg. produced slight sedation after about 3 hr.

In dogs, 10 mg./kg. given intraperitoneally produced decrease in general activity, lack of interest in surroundings, and mild loss of muscle tone after about 90 min. The dogs recovered in about 6 to 8 hr.

In monkeys 25 mg./kg. given intravenously produced sedation, and loss of pacing movements in the cage after about 1 hr. The monkeys preferred to lie down in the cage but did not sleep and could be easily approached by the observer. The effect lasted for about 5 hr.

Compounds 1433 and 1429 produced similar actions in animals. With compound 1433, onset of action was within 30 min., and lasted for about 6 hr. On oral administration, the effect of this compound was better than compound 1421.

Spontaneous Motor Activity in Mice

The spontaneous motor activity in adult mice was measured in spring mounted cages (Actograph-Jacquet). Movements were recorded graphically on smoked paper. A mouse was injected intraperitoneally with solvent and placed in the recording cage. The activity for the following 10 min was recorded. After this control observation, the mouse was injected with test compounds. Movements were recorded 1, 2, and 4 hr after injection. Test group received only solvent. Three mice per dose were tested at 20

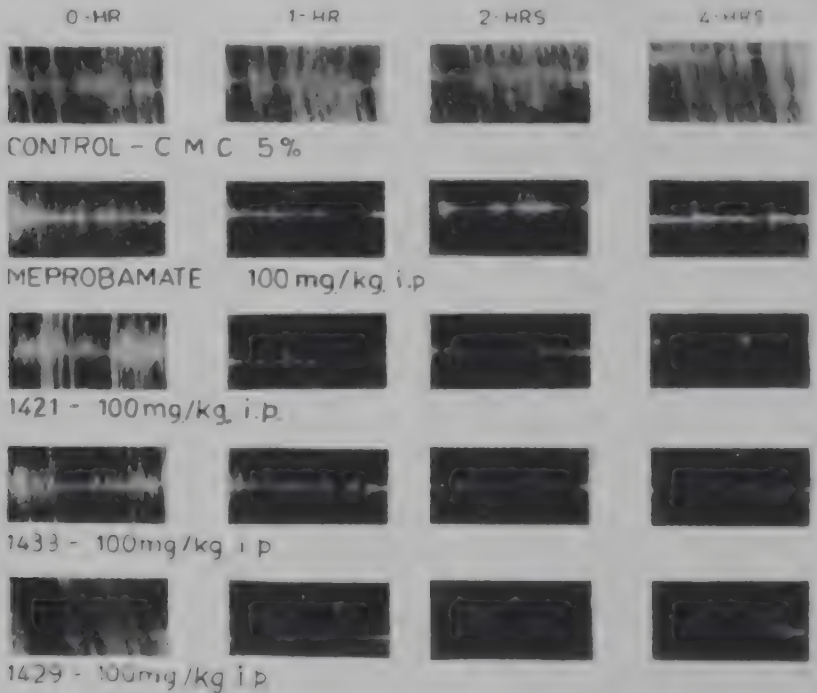


Fig. 2. Spontaneous motor activity (actographs).

TABLE 5—SPONTANEOUS MOTOR ACTIVITY

(Six mice in a group; Activity recorded for 10 min.)

No. of groups	Drug	Dose *mg./kg. i.p.*	Before	% Inhibition after the drug			Symptoms
				1hr	2 hr	4 hr	
3	Normal saline	0·1 ml./10 g.	885	2	12	38	Active, moving all over
3	Meprobamate	100	735	15	77	56	Decrease in activity. Sitting in a group; no ataxia, slightly relaxed, blepherospasm present.
3	1421	100	600	43	73	76	
3	1429	100	609	60	61	46	
3	1433	100	858	46	64	75	

TABLE 6—ORIENTATION HYPERACTIVITY IN MICE

(Six mice per group)

No. of groups	Drug	Dose *mg./kg. i.p.*	Total activity for 30 min. ±S.E.	% Inhibition
3	Normal saline	0·1 ml./10 g.	1797±167	..
3	1421	100	1023±175	43
3	1429	100	1129±148	37
3	1433	100	652±97	64

and 100 mg./kg. i.p. (Fig. 2). There was a marked decrease in activity with all the compounds. The action started in 1 hr and lasted for next 3 hr.

Spontaneous activity in mice was also studied by recording the total activity of a group of 6 mice in a photoelectric activity box ('Actophotometer' of Metro Industries, USA). The period of recording was 10 min. at the end of 1, 2 and 4 hr after drug administration. The control group received only vehicle (Table 5).

The movements were markedly diminished. The mice sat huddled in a group. Blepherospasm was marked. There was no loss of muscle tone or ataxia.

Orientation hyperactivity was studied in adult mice by the method of Cutting *et al.*[31]. The total activity for 30 min. was recorded. The recorded activity was compared to solvent treated controls and expressed as percentage inhibition (Table 6). Compound 1421 inhibited an activity

by 43 per cent, 1429 one of 37 per cent and compound 1433 one of 61 per cent. Meprobamate at a dose 100 mg./kg. showed 39 per cent inhibition.

Effect on Amphetamine-induced Hyperactivity in Mice

The stimulating effect of amphetamine (4 mg./kg. s.c.) on spontaneous motor activity in mice and the effect of the test compounds injected i.p. at a dose of 100 mg./kg. one hour prior to amphetamine was recorded by actophotometer. Total activity of each group (6 mice per group) was recorded for 10 min. (Table 7).

Amphetamine increased activity to 195 when control was taken as 100. Compound 1421 reduced this by 15 per cent, compound 1429 by 40 per cent and compound 1433 by 116 per cent; meprobamate at 100 mg./kg. reduced the activity by 32 per cent.

Oxygen Consumption

Oxygen consumption test was carried out as suggested by Cutting *et al.*[31]. Time required for consumption of oxygen from 4 cc. of air per mouse was recorded. Five mice were tested per compound before and 60 min. after administration of meprobamate and the test compounds in a dose of 100 mg./kg. i.p. (Table 8).

There was a decrease in oxygen consumption with compounds 1421 and 1433, resembling that obtained with meprobamate.

Amphetamine Toxicity in Mice

Adult albino mice in group of 10 [Burn and Hobbs][32] were placed in a cubical wire cage 16 cm. after receiving subcutaneously 14 mg./kg. of amphetamine sulphate. Other groups received in addition the test compound or the vehicle one hour prior to amphetamine. Deaths were counted in 24 hr (Table 9).

TABLE 7—AMPHETAMINE-INDUCED HYPERACTIVITY IN MICE

(Six mice per group)

No. of groups	Drug and dose *mg./kg. i.p.*	Amphetamine sulphate *4 mg./kg. s.c.*	Cumulative motor activity for 10 min. (expressed as per cent of control)	% Inhibition
3	Normal saline 0·1 ml./10 g.	..	100	—
3	Normal saline 0·1 ml./10 g.	Injected	195	—
3	1421, 100 mg.	Injected	180	15
3	1429, 100 mg.	Injected	155	40
3	1433, 100 mg.	Injected	79	116

TABLE 8—COMPARATIVE OXYGEN CONSUMPTION IN MICE

Drug and Dose *mg./kg. i.p.*	Seconds to consume 4 ml. of air (average of 10 measures)		Difference
	Before	After	
CMC (Control) 0·5%	180	190	+10
Meprobamate, 100 mg.	175	211	+36
1421, 100 mg.	191	234	+43
1429, 100 mg.	170	190	+20
1433, 100 mg.	175	223	+48

TABLE 9—AMPHETAMINE AGGREGATION TOXICITY

(Ten mice in each group; 1421, 1433 & 1429 injected alone showed no mortality)

Amphetamine 14 *mg./kg. s.c.*	Drug	Dose *mg./kg. i.p.*	% Mortality
Injected	Normal saline	0·1 ml./10 g.	100
Injected	Meprobamate	100	80
Injected	1421	100	90
Injected	1433	100	70
Injected	1429	100	80

d-Amphetamine sulphate produced 100 per cent mortality in aggregated mice in 24 hr. Compound 1433 showed some protection. With the test compounds injected alone, there was no mortality.

Hexobarbitone Sleep

White male rats weighing between 110 and 150 g. were injected with meprobamate and the test compounds (100 mg./kg. i.p.) followed 60 min. later by 100 mg./kg. of sodium hexabarbitone given i.p. The duration of loss of righting reflex (sleeping time) was recorded (Table 10).

Rectal Temperature

Effect of the test compounds on the rectal temperature of adult male rats recorded with a thermometer was studied for 4 hr. The fall in rectal temperature was noted with compound 1433 only. The fall in temperature started at one hour, reached lowest level at 2 hr and was maintained low for 4 hr.

Amphetamine Stereotypes

Amphetamine induced stereotypes movements in mice were studied as described by Lapin and Shchelkunov[33]. Amphetamine sulphate was injected intraperitoneally in a dose of 15 mg./kg. Under large dose of amphetamine the mice showed cessation of normal locomotor and prolonged

TABLE 10 HEXOBARBITONE SLEEPING TIME

Hexobarbitone sodium solution 10 mg./ml.; compounds 1421, 1433 and meprobamate potentiated hexobarbitone sleeping time at a significant level

Drug and dose *mg./kg. i.p.*	Hexobarbitone *mg./kg. i.p.* (90 min. after the drug)	No. of rats	Sleeping time, min. mean ± S.E.	'P' Value
Control	100	6	24 ± 0·84	
Meprobamate, 100 mg.	100	6	36 ± 1·60	*0·01
1421, 100 mg.	100	6	34 ± 3·30	*0·02
1429, 100 mg.	100	6	31 ± 5·30	..
1433, 100 mg.	100	6	32 ± 1·90	*0·01

*Significant

maintenance of a characteristic immobile posture with stereotype movements of the head and front limbs. Duration of absence of the normal locomotion was noted. Meprobamate and the test compounds reduced this period of abnormal movements by about 40 per cent (normal 150 min., with drugs 90 min.).

Effect on reserpine treated mice. Test compounds were injected intraperitoneally (100 mg. kg.) in adult white mice and 2 hr later reserpine 2·5 mg. kg. i.p. was injected and the mice were observed. The sedative action of reserpine was potentiated by all the test compounds.

Analgesic effect. The analgesic effect was investigated by a number of tests, viz. (a) hot plate method, (b) clip method, (c) rat tail flick method using analgesiometer (Davies[31]), and (d) Nilsen's[35] method using an electrical stimulus applied to mouse tail to produce squeak response.

No analgesic effect could be detected.

Anticonvulsant activity. Anticonvulsant activity of test compounds was studied using maximal electric shock test (MES) and metrazol seizure threshold test (Met. Test (Swinyard, Brown and Goodman[36]). Ten mice were used for each test and the tests were carried out 1, 2, 3 and 4 hr after administration of the test compounds (100 mg. kg. i.p.).

The test compounds failed to protect mice against MES and chemically induced seizures.

Conditioned avoidance response (CAR). CAR was established in rats using Cooks pole climbing technique (Cook and Weidley[37]). Under the effect of the compounds (20 mg. kg. i.p.) there was some inhibition of CAR and latency response to a conditioned stimulus was appreciably prolonged. The results were similar to meprobamate.

Acute toxicity. Acute toxicity was studied in white mice. The test compounds were injected i.p. and LD_{50} in terms of mg. kg. was calculated (Litchfield and Wilcoxon[38]). In all compounds LD_{50} was found to be greater than 1600 mg./kg.

Action on isolated intestine. Effect of the test compounds on isolated guinea-pig ileum was studied by usual method. The test compounds were added in a concentration of 10μg./ml. in a 30 ml. bath. No effect was observed on normal movements. Only compound 1433 showed some block of the acetylcholine induced spasm. The effect was reversible on washing.

Action on blood pressure and respiration. Carotid blood pressure was recorded in dogs, anaesthetized with pentobarbital sodium (35 mg./kg. i.p.). Test compounds were suspended in 0·5% CMC and injected i.p. in a dose of 6 and 100 mg./kg. Effect on blood pressure, carotid occlusion reflex and response to intravenous injections of adrenaline (1 μg./kg.), noradrenaline (1 μg./kg.), acetylcholine (1 μg./kg.) and histamine (1 μg./kg.) were noted. These observations were repeated at 30 min. interval for 4 hr after injection of the test compounds. No particular effect was noted.

Brain Acetylcholine

Male albino rats were injected with test compounds in a dose of 100 mg./kg. i.p. and brain acetylcholine was determined after 2 hr. Estimations were carried out on frog's rectus muscle. No significant change was noted.

Frog Rectus

Effect of test compounds on acetylcholine induced contraction of frog's rectus abdominis muscle was studied. There was no effect.

Antistress Effect

Effect on adrenal ascorbic acid and cholesterol in rats under standardized centrifugal stress applied in a vertical plain was noted (Pohujani *et al.*[39]). This stress depleted both adrenal ascorbic acid and cholesterol. The effect of test compounds and some other CNS depressants in blocking this effect is shown in Table 11.

Only chlorpromazine significantly inhibited the stress induced depletion of adrenal ascorbic acid and cholesterol.

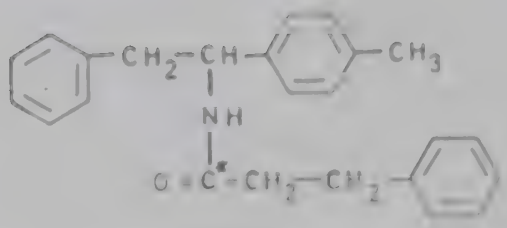
Subacute toxicity studies. Compound 1421 was administered in rats in a dose of 80 mg./kg. orally and i.p. for a period of 8 weeks. Body weight, haemogram, SGOT and SGPT studies, and histological studies failed to reveal any adverse reactions.

Resolution into *'l'* isomer. Compound 1421 was resolved into its *'l'* isomer; this did not show any enhanced activity.

Labeled studies. To study the absorption and metabolism of compound 1421, labeled isomer (XVI) has been synthesized.

Clinical Trials

(a) **Volunteers studies.** Pilot clinical trials with compound 1421



(XVI)
Labelled compound of code no.1421

were conducted in 37 volunteers. The drug was administered orally in a single dose of 200 to 300 mg. in capsules, and the effect was compared to placebo and 400 mg. of meprobamate, administered in identical looking capsules. Administration of compound was done in a random fashion, in a double blind technique. The volunteers were given a check list and were asked to check their reactions described in Table 12.

TABLE 11—EFFECT ON RAT ADRENAL ASCORBIC ACID AND CHOLESTEROL AFTER STRESS

Drug and dose *mg./kg.*	No. of animals	Adrenal ascorbic acid mg. per 100 g. of tissue, with S.E.)	Adrenal cholesterol (mg. per gm. of tissue, with S.E.
Normal	13	445·0 ± 4·5	33·65 ± 1·5
Control	16	252·0 ± 9·1	23·13 ± 0·61
Meprobamate, 100 mg.	6	290·6 ±21·8	23·07 ± 0·58
Chlorpromazine, 100 mg.	6	*404·0 ± 3·0 (P 0·01)	*29·87 ± 0·35 (P 0·01
1421, 100 mg.	6	272·94 ± 4·9	21·84 ± 0·95
1433, 100 mg.	5	278·29 ± 9·9	22·17 ± 0·34

*Significant

TABLE 12—CHECK LIST

Date:	Name:	Sl. No.:	Age:	Drug No.:	Sex:

Alertness	Salivation
Concentration	Heaviness of Head
Interest in Activities	Lethargy
Mood	Uncomfortable
Thinking	Action
Calmness	I. Onset
Drowsiness	II. Peak
Appetite	III. Duration
Tension	Remarks

TABLE 13—ANALYSIS OF RESULTS OF STUDY DONE WITH COMPOUND 1421, MEPROBAMATE AND PLACEBO IN HUMAN VOLUNTEERS

Drug and dose *mg.*	Number	Reactors	Non-reactors
1421			
200-300	37	13	24
Meprobamate, 400	37	14	23
Placebo	37	3	34

TABLE 14—CLINICAL TRIALS (COMPOUND 1421)

Number of Patients		25
Males	15	
Females	10	
Age: 20-60 Years		
Diagnosis: Anxiety Neurosis		
Duration of Illness: 6-36 months		

Each person received all three test compounds, in a random fashion on alternate days. Compound 1421 was equivalent to meprobamate in its general effects (Table 13). Both the drugs produced significant response as compared to placebo. No side reactions were reported.

(b) **Trials on patients with anxiety neurosis.** The trial was conducted at the Psychiatric Department of K.E.M. Hospital, Bombay. Twenty-five patients were included in the study (Table 14).

Diagnosis of anxiety neurosis was arrived at by 2 psychiatrists, examining the patients independently. The duration of illness varied from 6 months to 3 years. Only those patients were included, about whom both observers agreed on diagnosis. Attempts were made to study the social pathology, and each patient was given 'Rorschachs Test' and 'Taylor's Manifest Anxiety Scale'. The psychological tests confirmed the clinical diagnosis. Jackman's neurotic index was noted at the beginning and completion of the treatment (Table 15).

Each patient was assessed every week for improvement, and side effects. The drug was given orally in a dose of 100 mg. three times a day in the last week; 4 times a day in the second week; 5 times a day in the third week, and 200 mg. three times a day in the fourth week. Haemogram, urine examination, liver function tests, SGOT and SGPT were done at the beginning and end of trial. These tests did not reveal any adverse effects. The criteria of improvement are judged as shown in Table 16.

TABLE 15 - CLINICAL TRIALS (COMPOUND 1421)

Analysis of Jackman's neurotic index

No. of patients	Jackman's neurotic index		Remarks
	Beginning	End	
17	27 (14–36)	4 (0–8)	Improved
3	23 (22–24)	21 (20–22)	Not improved

TABLE 16—CRITERIA OF IMPROVEMENT

(*a*) Subjective Account of the Patient
(*b*) Objective Account from Patient's Relative
(*c*) Reduction in Jackman's Neurotic Index
(*d*) Two Independent Psychiatric Exams
Improved—50% and More Remission in Symptoms

TABLE 17—CLINICAL TRIALS (COMPOUND 1421)

Results	
Number of Patients	25
Discontinued	5
Number of Patients Completing four weeks of Treatment	20
Number of Patients Improved	17
Not Improved	3

Only those patients who showed 50 per cent and more remission in their symptoms were considered as improved. Five patients dropped out during the first two weeks, due to inability to attend. Of 20 patients who completed the four week trial, 17 patients improved. Three patients had 50 per cent and more remission of symptoms and 14 patients had 75 per cent and more remission. Three patients failed to respond (Table 17).

The patients were followed up for a period of 4 weeks after stopping the drug. Three patients had relapse of symptoms, during the 1st week after stopping the drug; 3 patients had relapse 2 weeks after stopping the drug; 6 patients maintained improvement for 4 weeks; 5 patients could not be contacted for follow-up.

One patient complained of itching after 2 weeks of drug administration. No other undesirable effects were observed.

DISCUSSION

Compounds of the series 1, 2-diphenylethylamines have been previously studied for their antimitotic activity in the treatment of malignancy. Gunn and Gurd reported CNS depressant activity in 1940. Goodson, Wiegud and Splitter reported analgesic activity in N-methyl derivatives of this series in 1946. However, none of these compounds were further studied for CNS activity.

Our studies of the present series demonstrated interesting CNS depressant activity. Compounds 1421, 1429 and 1433 produced sedation, and tranquillization in cats, dogs and monkeys. They also decreased the spontaneous activity in mice as seen by various tests. Action of hexobarbitone and reserpine were potentiated and amphetamine induced hyperactivity was counteracted. There was no significant effect on b.p., respiration and smooth muscle. These compounds did not possess any analgesic or anticonvulsant activity. The general profile of activity resembles that of minor tranquillizer like meprobamate and chlordiazepoxide. LD_{50} was more than 1600 mg./kg. (i.p.) in mice. In experimental animals the drug is not absorbed well orally, though some late absorption could be demonstrated. In human trials the drug was effective by oral administration. There was a latent period of 60 to 90 min., for the onset of the action even after parenteral administration. It is likely that the compounds may be undergoing biotransformation. This requires further studies on the absorption and metabolism of these compounds.

Compound 1421 which has been tried clinically both in volunteers and in patients suffering from anxiety neurosis, showed effects similar to that seen with other minor group of tranquillizers, like meprobamate. No adverse effects have been recorded during the 4-week period of administration. However, it is very difficult to form any definite opinion with such a small series and with only 4 weeks of drug administrations. Further work is being carried out in larger number of patients, comparing the drug to meprobamate in a double blind fashion.

Pharmacologically compound 1433 seems more promising for further work.

The clinical trials reported here have been conducted on compound 1421, as that was the first interesting compound studied in the series. It is contemplated to take up compound 1433 for human studies after the toxicity studies are completed.

REFERENCES

1. Lettre, H. & Albrecht, M., *Z. Physiol. Chem.*, **281** (1944), 133–39.
2. Hartwell, J. L., Shear, M. J., Johnson, J. M. & Kornberg, S. R. L., *Cancer Research Proc.*, **6** (1946), 489.
3. Gunn, J. A. & Gurd, M. R., *J. Physiol.*, **98** (1940), 422.

4. Tainter, M. L., Luduena, F. P., Lackey, R. W. & Seeler, E. N., *J. Pharmacol.*, **77** (1943), 317.
5. [illegible]
6. Dodds, E. C., Lawson, W. & Williams, P. C., *Nature*, **151** (1943), 614.
7. Dodds, E. C., *Br. med. Bull.*, **4** (1946), 88.
8. Dodds, E. C., Lawson, W., Simpson, S. A. & Williams, P. C., *J. Physiol.*, **104** (1945), 47.
9. McPhee, W. D. & Erickson, E. S. (Jr), *J. Am. chem. Soc.*, **68** (1946), 624.
10. Cilag Ltd, Swiss Patent No. 251299, July 16, 1948.
11. Martin, H. & Habicht, E., Ger., 955508, Jan. 3, 1957, Cilag Ltd.
12. Zaheer, S. H., Sattur, P. B. & Rao, P., *Ann.*, **691** (1966), 55.
13. Allen, C. F. H. & Barker, W. E., *Organic Syntheses*, Collective Vol. 2, John Wiley & Sons, Inc. New York, (1943), 156.
14. Strassman, H., *Ber.*, **1** (1889), 1229.
15. Farbenind, I. G., German Pat. No. 637384, 1936 (Oct. 29).
16. Hauser, C. R. & Morris, C. F., *J. org. Chem.*, **26** (1961), 4740.
17. Baumgarten, H. E. & Petersen, J. M., *J. Am. chem. Soc.*, **82** (1960), 459.
18. Ogiu, K., Furgimura, H. & Yamakawa, Y., *Yakugaku Zasshi*, **80** (1960), 283.
19. Rao, P. P., Ph.D. Thesis, Banaras Hindu University, Varanasi, India (1964).
20. Gabriel, S. & Herzberg, M., *Ber.*, **16** (1883), 2038.
21. Shopee, C. W., *J. chem. Soc.*, **33** (1930), 976.
22. Buck, J. S., *J. Am. chem. Soc.*, **54** (1932), 3661.
23. Helfer, L., *Helv. Chem. Acta*, **7** (1924), 945.
24. Max. P. J. M. Jansen, *Rec. Trav. Chim.*, **50** (1931), 291.
25. Miller, W. V. & Rohde, *Ber.*, **23** (1890), 1898.
26. William, Henry Perkin (Jr) & Robert Robinson, *J. chem. Soc.*, **91** (1907), 1084.
27. Ide, W. S. & Buck, J. S., *J. Am. chem. Soc.*, **59** (1937), 726.
28. Schlittler, E., *Ber.*, **66** (1933), 992.
29. Kindler, K. & Peschke, W., *Arch. Pharm.*, **272** (1934), 60.
30. Chiavarelli, S. & Jorio, M. A., *Gazz. Chim. ital.*, **86** (1956), 1054.
31. Cutting, W., Parkman, H., Read, G., Read, D., Cutting, J. & Furst, A., *Arch. int. Pharmacodyn.*, **121** (1959), 14–29.
32. Burn, J. H. & Hobbs, R., *Arch. int. Pharmacodyn.*, **113** (1958), 290.
33. Lapin, I. P. & Shchelkunov, E. L., Second International Pharmacological Meeting Volume I, p. 205.
34. Davies, O. L., Raventos, J. & Walpole, A. L., *Br. J. Pharmac.*, **1** (1955), 255.
35. Nilsen, *Acta. Pharmac. toxicol.*, **18** (1961), 10.
36. Swinyard, E. A., Brown, C. & Goodman, L. W. S., *J. Pharmac. exp. Ther.*, **106** (1952), 319.
37. Cook, L. & Weidley, E. F., *Ann. N.Y. Acad. Sci.*, **96** (1962), 315.
38. Litchfield, J. T. (Jr) & Wilcoxon, F., *J. Pharmac. exp. Ther.*, **96** (1949), 99.
39. Pohujani, S. M., Thesis for M.Sc. degree, University of Bombay, 1965.

Selective and Chronic Drug Access to Specific Regions of the Central Nervous System in Cats

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Two basic experimental requirements for assessment of drug actions upon loci of higher functional representation in the central nervous system are: (i) use of healthy, unanaesthetized, unrestrained animals chronically adapted to their laboratory environment; (ii) capability of selective access to target areas in the brain from either side of the blood-brain barrier.

These requirements have been approached by permanent implantation of cannulas and catheters in appropriate compartments of the cerebrospinal fluid (CSF) system and in blood vessels with known regional distributions.

Interpretation of responses to substances injected into the CSF depends upon specific site of drug deposition and upon volume distribution of the vehicle in relation to surrounding structures. We have reduced to routine practice the stereotoxic insertion into various CSF spaces in the cat of a puncture-cap cannula with a dead-space of less than 10 microlitres. Serial X-ray pictures were taken following single and multiple injections of radiographic contrast media at the different sites of cannulation. As little as 100 microlitres of solution injected into the anterior horn of the lateral ventricle, or 50 and 20 microlitres into the third and fourth ventricles, respectively, was visualized immediately in the foramina of Luschka connecting with the subarachnoid spaces. Injection of 50 microlitres into the posterior horn of the lateral ventricle delineated the entire ventricle as well as the foramen of Monro. Only 12 microlitres deposited in the base of the third ventricle served to define the hypothalamic cleft and infundibular recess. Radiopaque media did not enter the ventricular spaces when injected into the subarachnoid cisternae ambiens and magna. Evidence based upon progression and disappearance of contrast media injected intraventri-

cularly points to streaming flow of CSF allowing longest tolonra of injected substances in the ventral portions of the lateral and third ventricles.

Our efforts have been directed towards the elucidation of central drug actions concerned with the following forms of functional expressions: (a) emesis, (b) body temperature, (c) respiration, (d) blood glucose concentration, and (e) behavioral and toxic effects.

Emesis

Despite the fact that ablation of the emetic chemoreceptor trigger zone (CTZ) in the area postrema of the medulla oblongata abolishes the vomiting induced by a variety of substances, considerable variation in effective route of administration and character of emetic responses exists between the various agents. In the cat, apomorphine, morphine and epinephrine do not reliably elicit vomiting through systemic routes, yet they are effective with a short latency of the emetic response when injected into the cerebrospinal fluid. It is noteworthy that emesis is elicitable by these agents whether they find access to the receptor site from either the ventricular or subarachnoid CSF pools, especially in view of the demonstration that intracisternally injected media fails to enter the ventricular system. This means that the CTZ is available to stimulation from ventricular as well as subarachnoid exposure which is consistent with its morphological relationship to the rhombencephalic tela chorioidea.

The cardiotonic steroids and tetrodotoxin (puffer fish poison) exemplify another group of agents which are effective emetics with a more prolonged latency when administered systemically but which do not evoke vomiting when injected into the CSF. Regional vascular injections revealed, however, that the cardiotonic steroids were most effective as emetics through the intravertebral arterial route and least effective through the carotid artery by comparison with intravenous and intraportal injections.

Thus, in the case of the cardiotonic steroids, the emetic receptor elements are responsive to the agents from the vascular but not the ependymal side of the CTZ. These steroids, as well as tetrodotoxin, have been shown by others to have a selective blocking action on sodium transport in different tissue membranes.

Body Temperature

Pyrogenic effects of purified lipopolysaccharide (endotoxin) from *S. typhosa* and of staphylococcal enterotoxin were studied with the use of permanently implanted retroperitoneal thermocouples. Both toxins elicited emesis and fever when administered systemically but they elicited only fever when injected intraventricularly; neither the emesis nor the fever response was influenced by ablation of CTZ. Relative pyrogenic potency by ventricular and systemic routes was approximately 1000 : 1. The fever response to both agents is characteristically bimodel after intravenous injection and

unimodel after intraventricular injection. However, whereas tolerance to endotoxin developed after repeated intravenous but not after intraventricular administration, tolerance to enterotoxin was evident after repeated injection by either route. No cross-tolerance was demonstrable between endotoxin and enterotoxin. In contrast with the bacterial toxins, tetrodotoxin was remarkably potent as a thermoregulatory *paralyzant* when administered intraventricularly, but it gave only a weak hint of this capability when injected intravenously. Intracisternal injection of tetrodotoxin also failed to influence temperature regulation, which supports the contention that tetrodotoxin acts on a paraventricular structure in the forebrain.

Respiration

Respiratory failure due to tetrodotoxin is effected by neuromuscular blockade after systemic administration and by central paralysis after intraventricular injection of the substance. The ratio of intravenous to intraventricular dose requirement is approximately 100. Character of the central respiratory events as well as failure of intracisternal injection of tetrodotoxin to influence the respiration points to a selective action of the drug on a supramedullary locus.

Hyperglycemia

Intraventricular injection of morphine evoked hyperglycemia of similar magnitude and time course, but with only 1/50 of the dose requirement, as that elicited by intravenous administration of the drug. Morphine failed to elicit hyperglycemia after intracisternal injection. Morphine was at least as effective in eliciting hyperglycemia when injected intraventricularly in a small volume of solution that localized in the vicinity of the foramen of Monro as in larger volumes that distributed throughout the ventricular system. Ablation of the CTZ did not influence the hyperglycemic response. It is concluded from these results that morphine-induced hyperglycemia is evoked through an action of the drug on a receptor locus in the vicinity of the foramen of Monro.

Behavioral and Toxic Effects

Dissociation of central effects of morphine according to site of injection is well demonstrated by the following observations. Intracerebral administration elicited characteristic behavioral excitement but neither hyperglycemia nor emesis. Intraventricular injection elicited excitement, hyperglycemia and emesis. Intracisternal injection elicited emesis and excitement but not hyperglycemia. Intravenous injection elicited excitement and hyperglycemia but not emesis. In the case of tetrodotoxin, intraventricular injection elicited catalepsy and unpredictable pulmonary edema, whereas intracisternal and intravenous injection failed to elicit such effects. The cardiac glycosides were extremely noxious upon intraventricular

injection whereby they elicited seizures and pulmonary edema; such effects were not elicitable through intracisternal or intravenous administration.

In conclusion, we have endeavoured to show in this paper that qualitative as well as quantitative differences in central drug effects are revealed not only by a comparison of responses obtained through systemic and cerebrospinal fluid routes of administration but also by strict attention to discrete site of drug application within the cerebrospinal fluid system.

Sites of Action of Drugs in the Central Nervous System

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The actions of drugs on the central nervous system can be studied in a variety of ways. Thus, we can use biochemical techniques to monitor chemical changes in the brain when drugs are administered or we can follow neurophysiological changes and thirdly, we can use the techniques from experimental psychology to investigate the effects of drugs on behaviour. Our investigations have been largely concerned with studies of the effects of drugs on electrophysiological phenomena in the brain and the correlation of the changes induced with changes in behaviour. These studies have ranged from observations of effects on the EEG in intact animals to studies of the pharmacological properties of single neurones in the brain. A great deal of data has been derived from these studies and some of this will be briefly reviewed here.

The use of animal preparations with permanently implanted recording electrodes by means of which the effects of drugs on the electrical activity of the brain can be observed in unanaesthetized and freely-moving animals (Bradley and Elkes, 1953) has been useful for demonstrating that drugs have effects on, for example, the sleep-wakefulness continuum or arousal response and examples here are the barbiturates and central excitant drugs like amphetamine. These drugs produce changes in the electrical activity of the cerebral cortex which correlate well with the behavioral changes (Fig. 1). Such methods have also enabled a further analysis of the effects of drugs to be made on acute animal preparations and an approximate designation of their sites of action to the arousal mechanisms situated in the brain stem reticular formation (Bradley and Elkes, 1957). Furthermore, such experiments with chronic animal preparations have demonstrated that the correlation between electrocortical activity and the behavioral state in terms of wakefulness and sleep is not always maintained when pharmacological agents are administered. Thus, certain drugs which act on cholinergic

mechanisms in the brain produce changes in electrocortical activity which do not correlate with behaviour. For example, physostigmine causes a pattern of alertness to appear in the E.E.G but the animal is not alerted behaviourally and atropine induces a pattern resembling sleep but the animal is fully awake or even excited (Fig. 2). These observations point to the need for caution in the interpretation of electrophysiological data when they are studied in isolation.

Studies of the actions of drugs which do not have simple depressant or excitant actions on the central nervous system, particularly some of those used in psychiatry, such as the tranquillizers, show that their action may also be related to arousal mechanisms in the brain stem. Thus, chlorpromazine produces a reduction in motor activity and causes a state of indifference and unresponsiveness; this behavioral state correlating well with the changes in electrocortical activity (Fig. 3).

The results of experiments with the chronic animal preparations together with those from acute experiments suggested therefore that the sites of action of certain drugs might be related to arousal mechanisms in the brain stem. A study of the effects of drugs on arousal responses was therefore indicated.

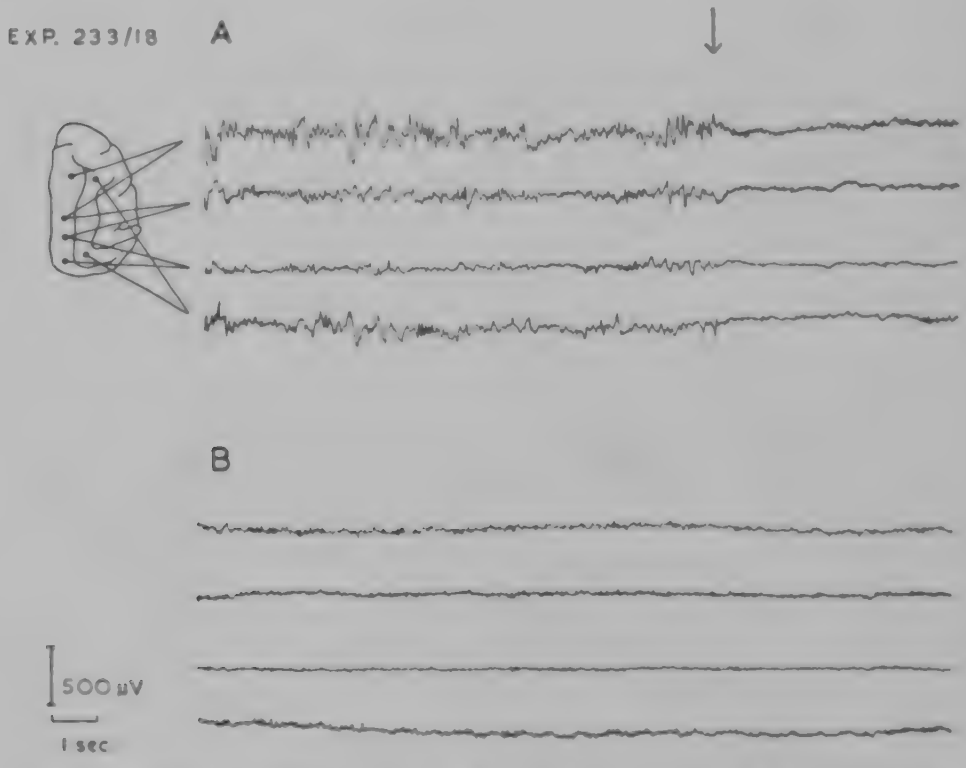

FIG. 1—EFFECT OF *dl*-AMPHETAMINE ON ELECTROCORTICOGRAM OF AN UNANAESTHETIZED CHRONIC CAT PREPARATION [A, Normal control record showing an arousal response to a sensory stimulus 'S'; B, Twenty minutes after the injection of 2·3 mg./kg. *dl*-amphetamine sulphate (i.p.)]

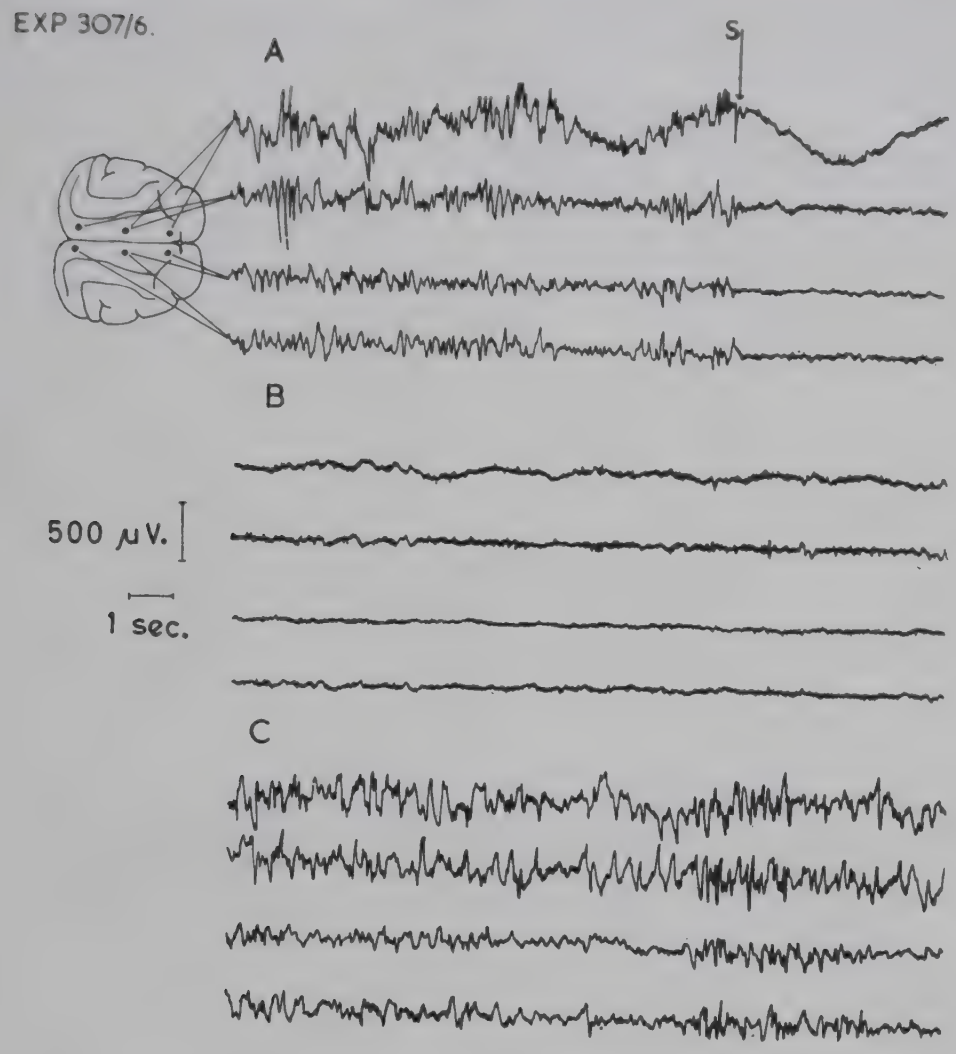

FIG. 2—EFFECTS OF PHYSOSTIGMINE AND ATROPINE ON THE ELECTROCORTICOGRAM OF AN UNANAESTHETIZED CHRONIC CAT PREPARATION [A, Normal control record showing an arousal response to sensory stimulus 'S'; B, Ten minutes after 0·08 mg./kg. of physostigmine sulphate (i.p.); C, Twenty minutes after the subsequent injection of 3·0 mg./kg. of atropine sulphate (i.p.)]

Arousal responses were produced in two ways: firstly, by direct electrical stimulation at 300 c./s. of the brain stem reticular formation by means of stereotactically orientated stimulating electrodes, using the methods which Moruzzi and Magoun (1949) used in their original experiments; secondly, by afferent stimuli, in our case a sound produced by a loudspeaker placed close to the head of the animal. The thresholds were determined before the administration of any drugs, as control levels, either as the voltage applied to the stimulating electrodes in the brain stem or to the loudspeaker. The drugs were then administered in incremental quantities and the threshold re-established after each injection. They were plotted as the percentage change from the control value against the dose of the drug. With a barbiturate, such as pentobarbitone, the threshold for arousal to brain stem

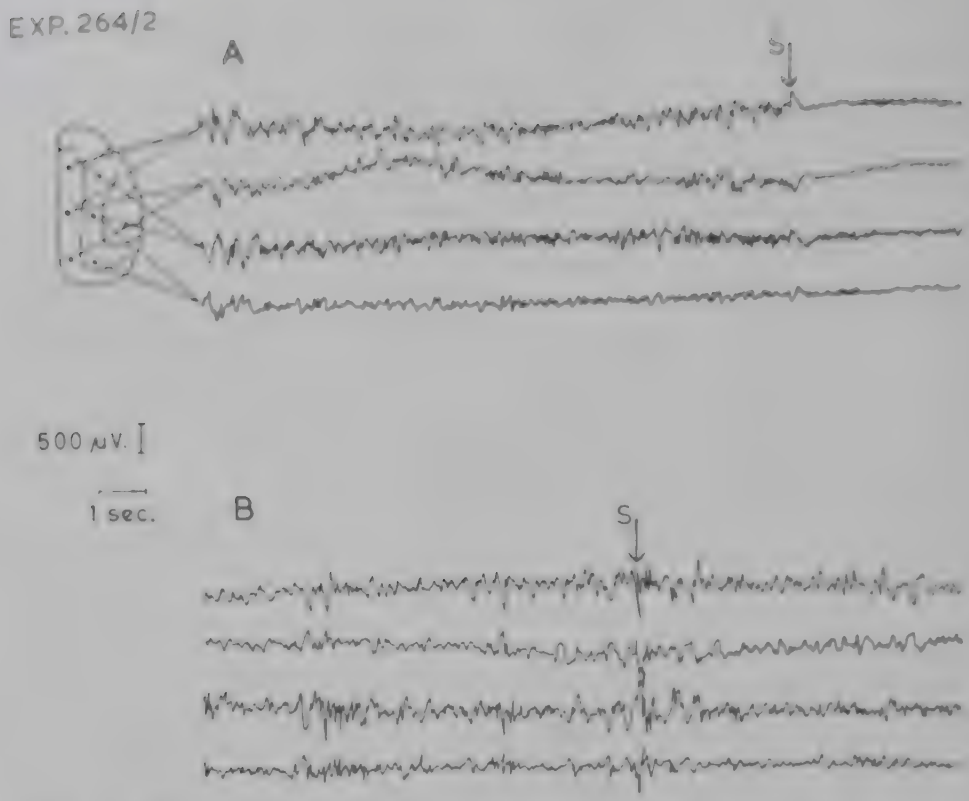

FIG. 3—EFFECT OF CHLORPROMAZINE ON ELECTROCORTICOGRAM OF AN UNANAESTHETIZED CHRONIC CAT PREPARATION [A, Normal control record showing arousal response to sensory stimulus 'S'; B, Twenty minutes after the injection of 15·0 mg./kg. of chlorpromazine (i.p.) showing absence of response to the same sensory stimulus]

stimulation increased rapidly as the dose was increased and arousal responses became completely blocked even with relatively small quantities (8–10 mg./kg.) (Fig. 4B). In the case of chlorpromazine, however, there was only a slight increase in the threshold for arousal to brain stem stimulation, even with large doses (Fig. 4A). On the other hand, this drug had a very pronounced depressant effect on afferent-induced arousal responses and the threshold rose very steeply. We also measured the threshold for click responses recorded from the auditory area of the sensory cortex and found that this threshold was unchanged when chlorpromazine was given. It was noticed that the dose level (1·5–4·0 mg./kg.) at which the most marked change in the threshold for auditory-induced arousal occurred, corresponded to the dose which, in the intact animal with chronically implanted recording electrodes, produced a state of indifference and unresponsiveness to sensory stimuli of all kinds.

Similar experiments were performed with central excitant drugs such as amphetamine and the hallucinogenic compound D-lysergic acid diethylamide (LSD 25). Amphetamine produced a fall in the threshold for arousal to stimulation of the brain stem as the quantity of the drug was increased,

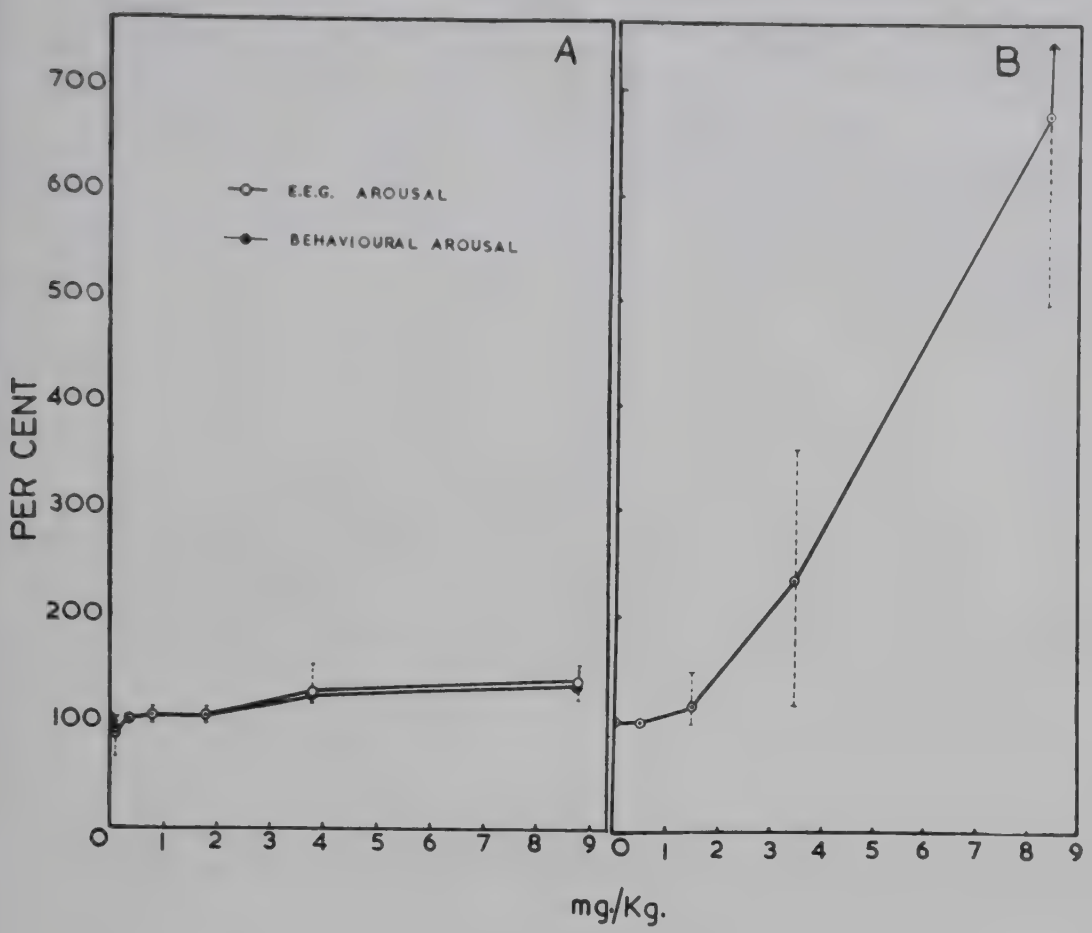

FIG. 4—EFFECTS OF INCREMENTAL DOSES OF CHLORPROMAZINE AND PENTOBARBITONE ON AROUSAL PRODUCED BY ELECTRICAL STIMULATION OF THE BRAIN STEM RETICULAR FORMATION [A, Chlorpromazine; B, Pentobarbitone. The mean value of the percentage change in threshold has been plotted against the dose of drug in each case]

until eventually complete alertness was reached (Fig. 5). There was a similar fall in the threshold for afferent-induced arousal. This supported the earlier hypothesis that amphetamine has a direct excitant action on the arousal mechanisms located in the reticular formation (Bradley and Elkes, 1953; 1957). There was no significant effect on the threshold for the click responses at the auditory cortex.

In the case of LSD 25, the threshold for arousal produced by brain stem stimulation was unchanged in doses up to about 50 μg./kg., which are the order of doses which produce excitation, increased alertness in the EEG and behavioral alertness in the intact animal. If much larger doses are used, there is depression and an increase in the threshold. We feel that doses of this drug below about 20 μg./kg. are the ones which produce effects in animals which are comparable to some of the effects observed in man, particularly increased responsiveness of the EEG (Bradley, Elkes and Elkes, 1953). If we look at the effect of this drug on afferent-induced arousal,

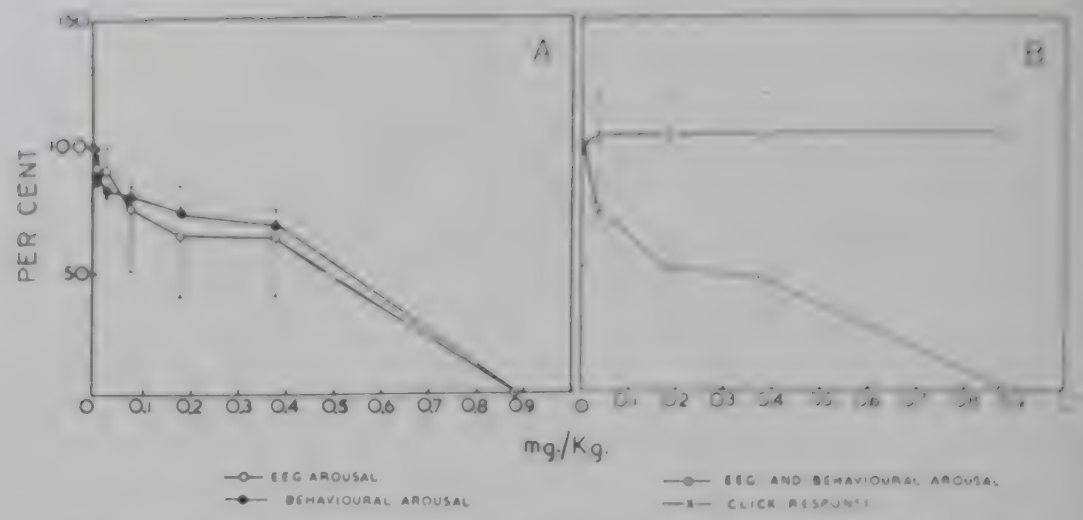

FIG. 5—EFFECTS OF INCREMENTAL DOSES OF *dl*-AMPHETAMINE ON AROUSAL RESPONSES [A, Arousal produced by direct electrical stimulation of the brain stem reticular formation; B, Arousal produced by auditory stimulation]

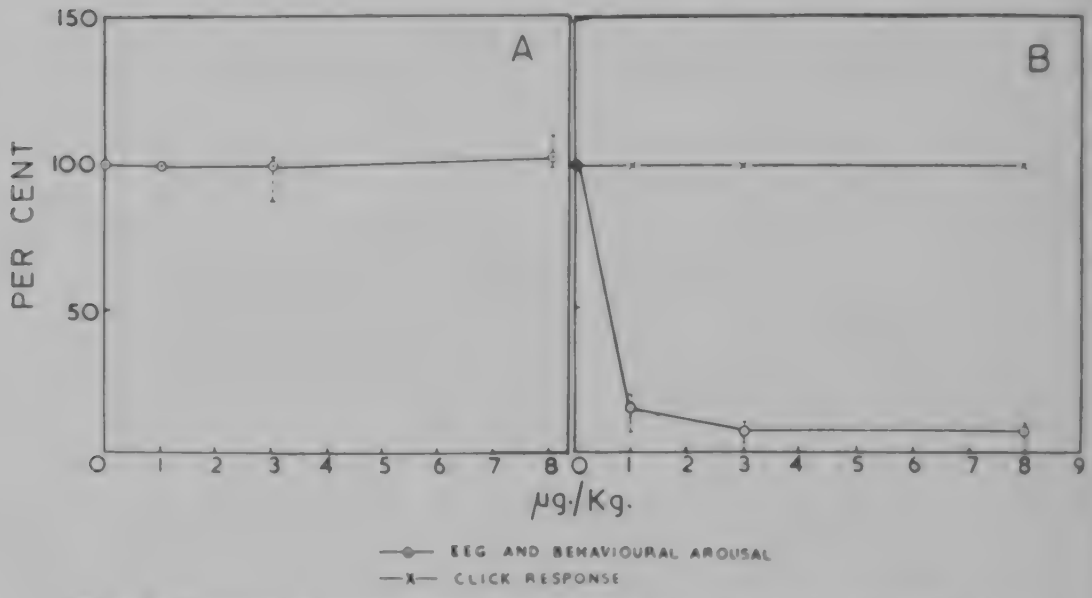

FIG. 6—EFFECTS OF INCREMENTAL DOSES OF LSD 25 ON AROUSAL RESPONSES [A, Arousal produced by electrical stimulation of the brain stem reticular formation; B, Arousal produced by auditory stimulation]

it can be seen that there is a marked fall in this threshold and at an extremely low dose level (Fig. 6). In other words, arousal is produced by a much lower level of stimulation, and this is consistent with qualitative observations on animals which had been given LSD 25 and which were found to be more responsive to sensory stimuli. Again, the threshold for click responses in the auditory cortex was unchanged.

From the experimental findings outlined above it can be postulated that drugs like the barbiturates have a direct depressant action on the

brain stem arousal mechanisms in the reticular formation and there is a considerable volume of evidence from other sources to support this. Amphetamine also has a direct action on the brain stem mechanisms, but in this case it is excitatory. Chlorpromazine and LSD 25, which produce little or no change in the threshold of the response from the sensory pathway itself, but a marked change in the threshold for afferent-induced arousal, are suggested as having actions that are related to the collateral input from the sensory pathways. In the case of chlorpromazine, this is a depressant action and in the case of LSD 25 it is an excitatory or facilitatory action.

These results demonstrate the importance of considering environmental influences when studying the effects of drugs, a factor which is often neglected in animal experiments. There are also other factors which needed some consideration. For example, although the effects of chlorpromazine on afferent-induced arousal were consistent with previous observations, they could equally well be explained by the process of habituation. This is a process by which the subject learns not to respond to stimuli which have no biological significance for it. It has been shown to occur for arousal responses by Sharpless and Jasper (1956) and for sensory-induced arousal at threshold levels, it occurs very rapidly. This can be demonstrated by plotting the threshold for afferent-induced arousal, using an intact animal with implanted electrodes, and presenting the same stimulus, a sound, repeatedly at threshold level. After a while the response disappears and cannot be restored unless the intensity is increased still further until eventually the arousal response to this particular stimulus is lost. If the stimulus is changed however, the response returns. This effect can be overcome by using positive conditioning in which the stimulus is paired with an electric shock applied to the animal during a training procedure. Not only is a lower level of threshold achieved, but its level is stabilized. The unreinforced sound stimulus can now be presented for between 30 and 40 trials, before the threshold begins to rise and the response is eventually extinguished. If at this point it is reinforced once more by applying the shock, the threshold remains unchanged for a much longer time. Here we have a means of obtaining a stable level of threshold and overcoming the effects of habituation.

An experiment was carried out with chlorpromazine in which two auditory stimuli were used to evoke arousal responses. One was non-conditioned and the second one had been conditioned by the method described above. Chlorpromazine was then given and it was found that the threshold for both of these stimuli increased in much the same way (Fig. 7). This suggested that here we were dealing with a genuine depressant effect of the drug on afferent-induced arousal, and that it was not masked by habituation (Key and Bradley, 1960). However, the effects of the drug and of habituation are to some extent inter-related, as will be shown.

A similar experiment was performed with LSD 25, but in this case three separate afferent stimuli were used and these were sounds at different

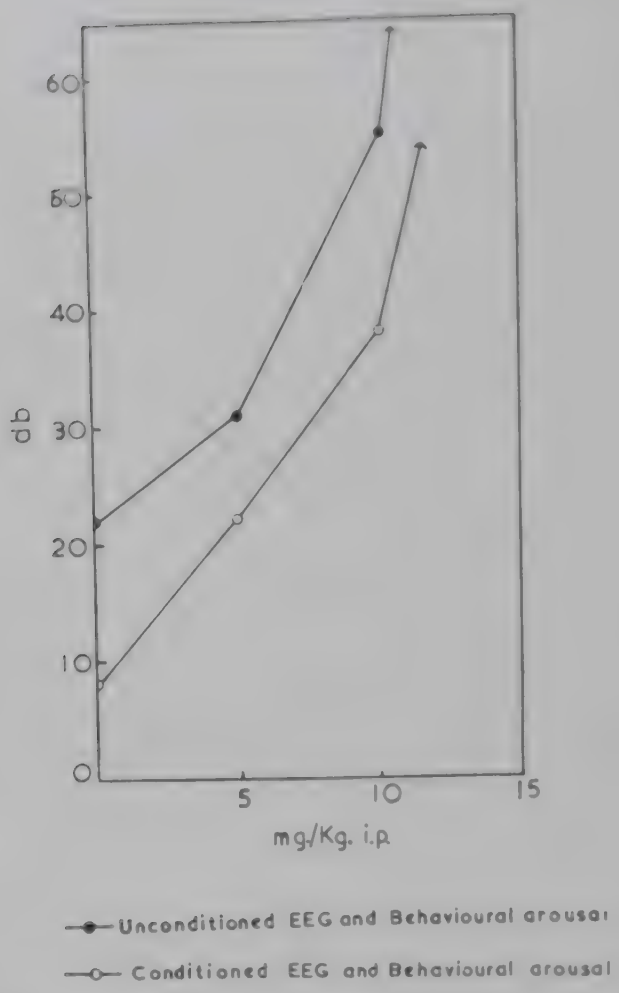

FIG. 7—EFFECTS OF INCREASING DOSES OF CHLORPROMAZINE ON THE THRESHOLD FOR AROUSAL PRODUCED BY CONDITIONED (OPEN CIRCLES) AND NON-CONDITIONED (SOLID CIRCLES) AUDITORY STIMULI [Intensity of the stimulus has been plotted in decibels]

frequencies (Fig. 8). The first was non-conditioned, and was presented to the animal only twice before the drug was given to avoid any habituation. After administration of the drug, the threshold for this stimulus was lowered by about the same amount as in the previous experiments with non-conditioned stimuli. The second stimulus had been previously conditioned so that it was at a lower level of threshold before the drug was given, and in this case no change occurred. The third stimulus was presented repeatedly to the animal until it was fully habituated before the drug was given, i.e. the response was lost completely. LSD 25 was then given and the response to this stimulus returned, not at its original level, but at a lower level corresponding roughly to the thresholds for the other two stimuli.

Thus, the effect of LSD 25 was not only to lower the threshold for afferent-induced arousal, but to overcome the effects of habituation. In this the drug appeared to stimulate the effects of positive conditioning, whilst

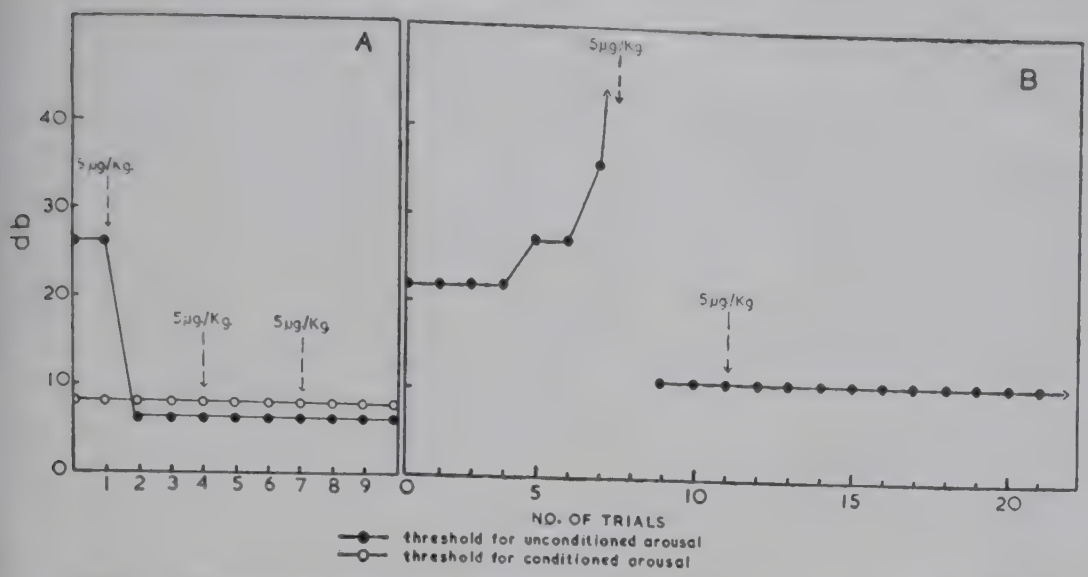

FIG. 8—EFFECT OF LSD 25 ON THE THRESHOLD FOR AROUSAL [A, Produced by conditioned (open circles) and non-conditioned (solid circles) auditory stimuli; B, To an auditory stimulus following habituation. The stimuli used in this experiment were tones of different frequencies]

chlorpromazine produced effects on these responses very similar to those which occur during habituation. It can be postulated that these two drugs, chlorpromazine and LSD 25 produce alterations in the significance level of sensory stimuli, and that these alterations are opposite in direction, being similar to the changes which occur during positive and negative conditioning (Key and Bradley, 1960). This is if we regard habituation as being a process of negative conditioning. Moreover, it appears that chlorpromazine lowers the significance level of sensory stimuli to a level at which no responses occur, as in habituation, and LSD 25 appears to raise the significance level of all stimuli to a level normally attained by positive conditioning. However, these experiments were performed with auditory stimuli using tones of different pitch for the different stimuli and one explanation for the effects observed could be that there was a change in the animal's ability to discriminate between the different sounds.

A study has therefore been made in which the effects of these two drugs on sensory discrimination and sensory generalization have been examined (Key, 1961). It is not necessary to describe the results of these experiments in detail, as they were largely negative. It was found that both chlorpromazine and LSD 25 had only slight effects on sensory discrimination as far as the parameters of stimulation were concerned, and that these could not have accounted for the effects which had been obtained in the previous experiments. The effects on sensory generalization were interesting because they showed a shift in the generalization curve without any alteration in its slope, so that there was no change in the rate of generalization. The experiments were carried out with a conditioned avoidance response; the animals

were trained to cross a barrier when a 600 c./s. note was sounded. After training had been completed, i.e. when the animals gave a 95 per cent correct response level, the generalization was determined by presenting different tones in random sequence and the extinction of the response was measured, i.e. the number of times the stimulus at any particular frequency had to be repeated before the response was lost. This was done for animals given control injections of normal saline and with animals given LSD 25 and chlorpromazine. These two drugs produced approximately equal and opposite shifts of the generalization curve. Chlorpromazine enhanced extinction at all frequencies, including the one to which the response was conditioned, and the response was extinguished much more quickly after this drug had been given, whilst LSD 25 retarded extinction. Thus, a greater number of presentations was required to extinguish the response at each frequency after LSD. However, the generalization curve itself, in so far as its shape is concerned, was not altered by either drug.

In a further experiment with these two drugs [Key, 1961], the rates of habituation of arousal responses were determined at different stimulus intensities by measuring the number of presentations of a given sensory stimulus, again using auditory stimuli, required for complete habituation of the arousal response. With control injections of normal saline, it was found that as the stimulus intensity was increased, so the number of trials for extinction of the response was correspondingly greater. After the administration of chlorpromazine, in two different dose levels, the response extinguished very quickly, even with high stimulus intensities, i.e. the number of presentations before extinction occurred was small. With LSD 25, the opposite effect occurred and a large number of presentations was required for extinction even at low stimulus intensities. Here again, we have chlorpromazine potentiating the effects of habituation on arousal responses so that the response is lost very quickly and conversely, LSD 25 retarding this process.

It seems likely therefore, that the actions of these two drugs are intimately connected with the neurophysiological processes regulating the significance level of environmental stimuli in relation to the previous experience of the animal. It would seem probable that LSD 25 does not alter the ability of the animal to discriminate between the physical parameters of the different stimuli but that the significance of one particular stimulus is more readily generalized to others previously without attentive or arousal value.

We believe that these processes may be occurring at the brain stem level where we have suggested that the site of action of these drugs lies. Their actions are known to be closely related to influences from the collateral sensory inflow into the reticular formation, and whilst these collaterals have been demonstrated to exist from below the level of the inferior colliculus to as far forward as the posterior part of the thalamus, it would be interesting

to know whether the drugs have a uniform effect at all levels of the collateral system.

Recent experiments (Key, 1965) have shown that whereas the effects of the barbiturates are uniform, those of chlorpromazine are not. Thus, arousal responses produced by electrical stimulation at different levels within the auditory pathway showed similar increases in threshold for all stimulation points with increasing doses of pentobarbitone, but chlorpromazine produced a differential effect depending upon the position of the stimulating electrode; the rise in threshold produced by chlorpromazine was greater for stimulation points below the level of the inferior colliculus than it was for more rostral placements. These results suggest that there may be a differential sensitivity to drugs at different levels of the collateral input into the reticular formation.

These experiments demonstrate how a combination of electrophysiological and behavioral techniques can assist in the analysis of the actions of drugs which modify the response of the organism to its environment, and to relate these actions to the neurophysiological mechanisms responsible for the filtering and integration of sensory information, and for the processes of conditioning and habituation.

In order to analyse the mechanisms involved in drug action on the CNS it is essential to know something about the pharmacological properties of the individual neurones in the brain, and to this end we have been studying the effects of various substances applied locally to the surface of the neurone by iontophoresis, whilst the electrical discharges of the neurones were recorded by means of microelectrodes. For this investigation we have used multi-barrelled microelectrodes, one barrel of which is used for recording the activity of the neurone, another barrel contains saline for passing a current equal to that used for iontophoresis, and the remaining three barrels of a five-barrelled microelectrode contain active substances. Such studies can serve two purposes. First, to determine the nature of synaptic transmitters in the brain, and secondly to determine whether substances with known actions on the central nervous system produce their effects by direct action on the neurones, or through some other indirect mechanisms. Most of the work which we have done in this field so far has been concerned with the analysis of the pharmacological properties of brain stem neurones and has been directed mainly towards the analysis of the actions of likely candidates for synaptic transmitters in this region of the brain (Bradley and Wolstencroft, 1965).

Of the centrally active drugs examined, LSD 25 and amphetamine have produced the most puzzling results so far. Both of these drugs had inhibitory actions as far as the activity of single neurones was concerned. The drugs did not influence the activity of all neurones on which they were tested; however, LSD 25 inhibited approximately 31 per cent (Fig. 9) and amphetamine 47 per cent. The action of LSD 25 was sometimes

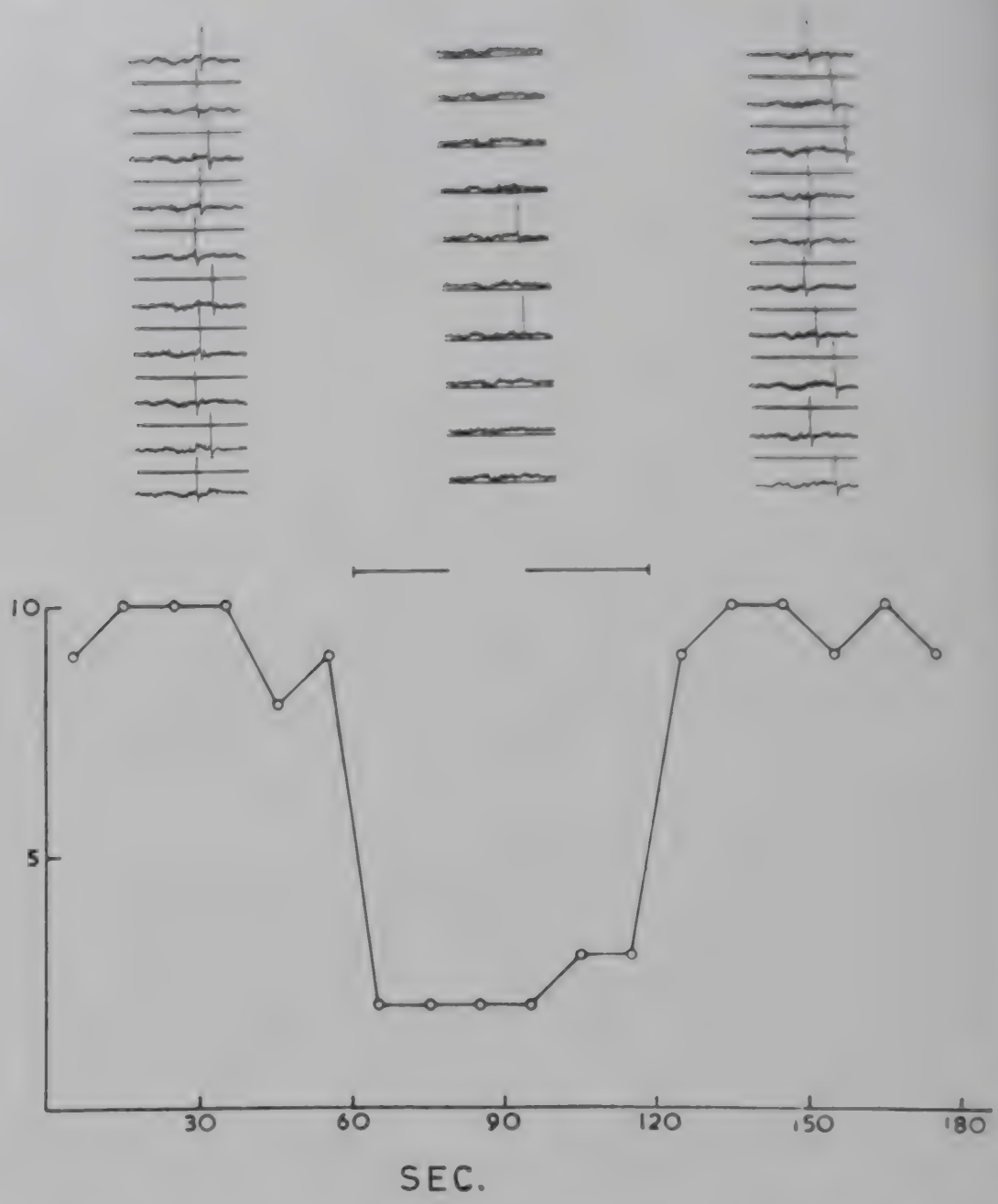

FIG. 9—EFFECT OF IONTOPHORETICALLY APPLIED LSD 25 ON THE ACTIVITY OF A NEURONE IN THE BRAIN STEM [Upper record shows oscillograph sweeps triggered at 1 sec. intervals before, during and after application of LSD 25. The graph shows the response frequency plotted for successive 10 sec. periods. LSD was applied with a current of 100 nA.]

prolonged and the activity did not return to its previous rate for perhaps some five to ten minutes after the application, whereas amphetamine did not show this prolonged action. On the other hand, when amphetamine was injected intravenously it was found to have an excitant action on the discharge rate of neurones in the reticular formation. The inhibitory effects

of these two drugs are in contrast to the inhibition of brain stem neurones produced by pentobarbitone, which inhibited the activity of all the neurones on which it was tested, but with varying sensitivity.

The results obtained with amphetamine and LSD 25 are puzzling and difficult to explain. One possibility which we must consider is that when applied directly these substances may have a local anaesthetic action and so cause inhibition of neuronal activity. If this was the case however, we could expect them to affect the activity of all the neurones on which they were tested. We must also consider the fact that when these drugs produce their usual effects on the central nervous system, they are administered orally or intravenously, and it is therefore possible that they may be changed in some way before they reach the brain, or before they reach their active sites. However, in the case of LSD 25, its very high potency points to a highly selective action and this might well be on individual neurones. In the case of chlorpromazine we have been unable to obtain any satisfactory results with application by the iontophoresis method, but when given intravenously, the reactivity of single neurones in the brain stem reticular formation is modified (Fig. 10). Thus, not only is the spontaneous

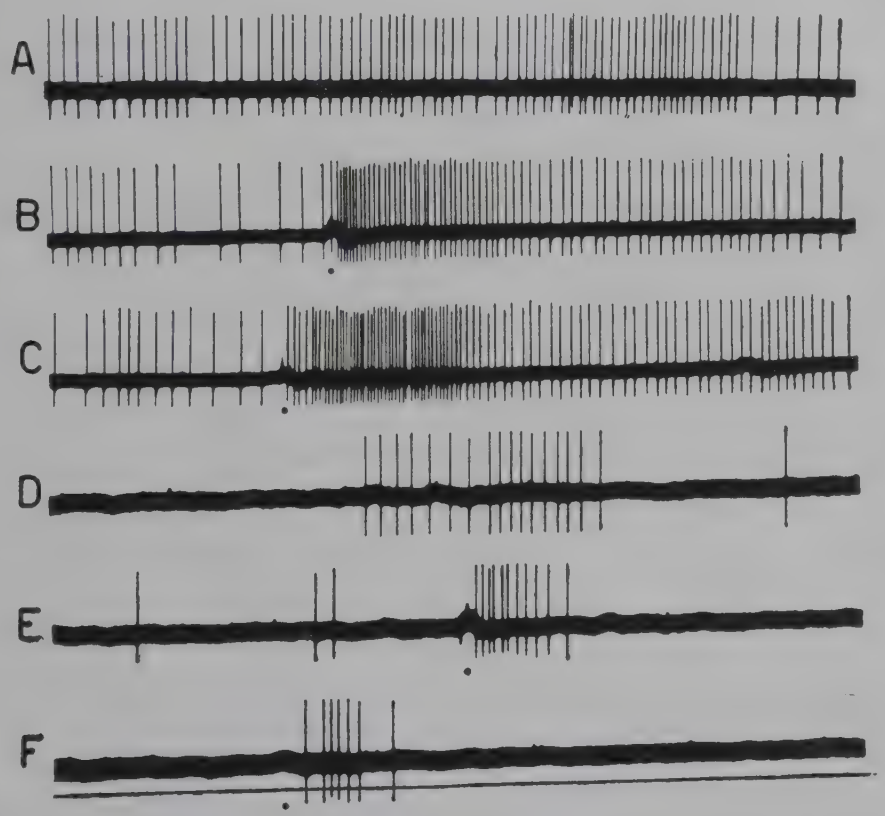

FIG. 10—EFFECT OF CHLORPROMAZINE INJECTED INTRAVENOUSLY ON THE ACTIVITY OF A NEURONE IN MESENCEPHALIC RETICULAR FORMATION [A, Spontaneous discharge; B, Response to tapping the right anterior leg; C, Response to trigeminal stimulation; D, Spontaneous discharge after chlorpromazine (2·0 mg./kg.); E & F, Responses to the same stimuli as B & C after chlorpromazine]

activity of the neurones reduced following administration of chlorpromazine, but with neurones showing convergence responses the responses to sensory stimuli are also considerably depressed. This is in keeping with the idea of a depressant action of this drug on the collateral sensory input into the brain stem reticular formation (Bradley, 1958).

It would be interesting to speculate further about some of these findings, particularly the apparent inhibitory actions of amphetamine and LSD 25, but we feel that this would be inadvisable at this stage, when the iontophoretic technique is still in its infancy and future experiments using these methods will, we hope, throw further light on some of these problems.

REFERENCES

1. Bradley, P. B., Psychotropic Drugs, Elsevier Publishing Company, Amsterdam, (1958), 207.
2. Bradley, P. B., *Desafferentation Experimentelle et Clinique*, Georg & Cie, Geneva, (1962), 37.
3. Bradley, P. B. & Elkes, J., *Electroenceph. clin. Neurophysiol.*, **5** (1953), 451.
4. Bradley, P. B. & Elkes, J., *J. Physiol.*, **120** (1953), 13.
5. Bradley, P. B. & Elkes, J., *Brain*, **80** (1957), 77.
6. Bradley, P. B., Elkes, C. & Elkes, J., *J. Physiol.*, **121** (1953), 50.
7. Bradley, P. B. & Key, B. J., *Electroenceph. clin. Neurophysiol.*, **10** (1958), 97.
8. Bradley, P. B. & Key, B. J., *Br. J. Pharmac.*, **14** (1959), 340.
9. Bradley, P. B. & Wolstencroft, J. H., *Br. med. Bull.*, **21** (1965), 15.
10. Key, B. J., *Psychopharmacologia*, **2** (1961), 352.
11. Key, B. J., *Nature*, **190** (1961), 275.
12. Key, B. J., *Electroenceph. clin. Neurophysiol.*, **18** (1965), 670.
13. Key, B. J. & Bradley, P. B., *Psychopharmacologia*, **1** (1960), 450.
14. Moruzzi, G. & Magoun, H. W., *Electroenceph. clin. Neurophysiol.*, **1** (1949), 455.
15. Sharpless, S. & Jasper, H., *Brain*, **79** (1956), 655.

Structure-Activity Relationship of Some Substituted Diaminopyridines

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In an extended programme for the synthesis of deazapurine nucleosides[1] a number of diaminopyridines were prepared which, on pharmacological screening, were found to possess marked vasopressor and antibarbiturate activity. These observations led to the synthesis[2] of a variety of diaminopyridines with substituents on the amino groups and in the pyridine nucleus. These diaminopyridines were also cyclized to the corresponding imidazo- and triazolo (4, 5-b) and (4, 5-c) pyridines. The various types of compounds thus synthesized are given in Fig. 1.

The pharmacological activity of aminopyridines is described in Table 1. The vasopressor, CNS and autonomic actions of 2- and 4-aminopyridines[3,4] were greatly enhanced by the introduction of an additional amino group in the 3-position; 3, 4-diaminopyridine[5] was the most active of the diaminopyridines.

The disappearance of the individual actions of 2- and 4-aminopyridines in 2, 4-diaminopyridines was most surprising. This could be due either to the competition between 2- and 4-amino groups for the same bioreceptors or due to the binding of the molecule at the 'sites of loss'.

The results of the pharmacological screening of some of the other representative compounds are given in Table 2. In general, substituted 2, 3-diaminopyridines (I) and 3-substituted imidazo- and triazolo (4, 5-b) pyridines (II and III) showed central nervous stimulant action. Thus 3-β-diethylamino-ethylimidazo (4, 5-b) pyridine (9) and its 6-nitro or 6-methoxycarbonyl derivatives (10 and 11) showed signs of stimulation in the gross behaviour and antagonized barbiturate induced hypnosis in mice. The 6-methoxycarbonyl compound (11) in addition showed respiratory stimulant action, which was more marked in the corresponding phenylethyl compound (12) and also in 3-β-phenylethyl-6-carbamoylimidazo (4, 5-b) pyridine (13). On the other hand, substituted 3, 4-diaminopyridines (IV)

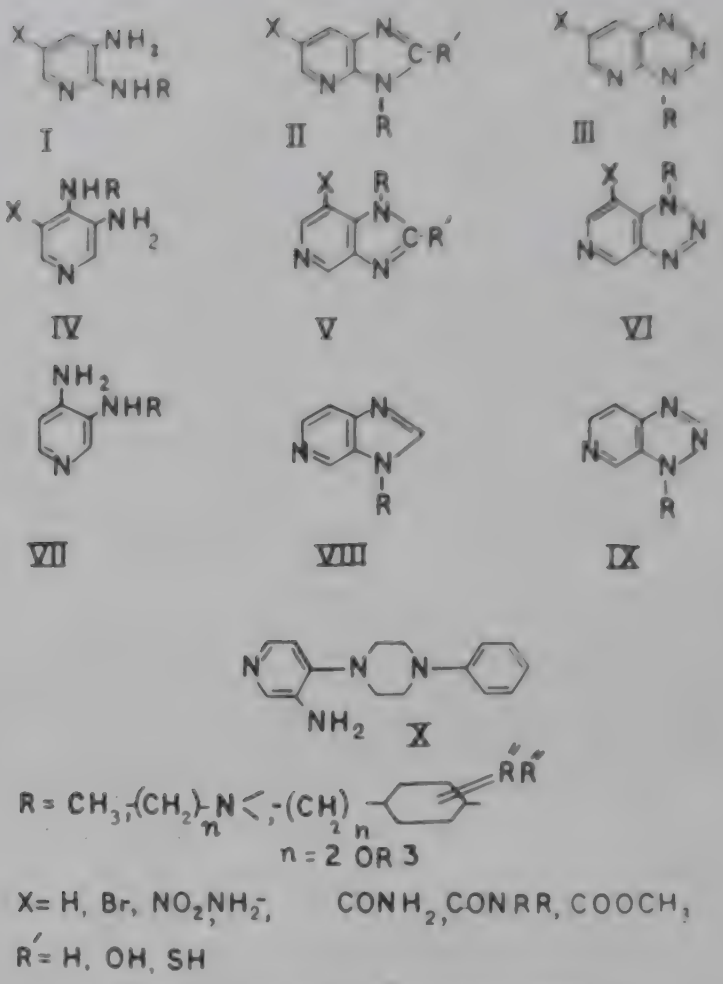


FIG. 1 — TYPES OF COMPOUNDS SYNTHESIZED

and 1-substituted imidazo- and triazolo (4, 5-c) pyridines (V and VI) showed depressant action. Thus 4-β-phenylethylamino-3-aminopyridine (15) showed signs of depression in the gross behaviour, produced a vasodepressor response and also blocked pentylenetetrazole and electroshock induced convulsions. Similarly, 1-β-phenylethyltriazolo (4, 5-c) pyridine (16) and 1-β-N'-piperidylethylimidazo (4, 5-c) pyridine (17) potentiated barbiturate induced hypnosis in mice. The latter compound (17) showed marked hypotensive and respiratory stimulant activity in dogs and also potentiated acetylcholine induced contractions in isolated guinea-pig ileum. When the 4-amino group in 3, 4-diaminopyridine was made the part of a cyclic system as in 1-(3-amino-4-pyridyl)-4-phenyl piperazine (18) it showed a stimulant action and blocked electroshock, pentylenetetrazole and strychnine induced convulsions.

During the course of these investigations, a patent[6] to CIBA group of workers appeared, claiming analeptic activity for N-substituted imidazo (4, 5-b) and (4, 5-c) pyridines (II and V). While this study agreed with the claimed analeptic activity of the former, it showed that the corresponding imidazo- and triazolo (4, 5-c) pyridines have a depressant action.

TABLE 1—PHARMACOLOGICAL ACTIVITY OF AMINOPYRIDINES

S. No.	Pyridine derivatives	Approx. LD_{50} (mice) *mg./kg. i.p.*	Gross effect (mice)	Effect on barbiturate hypnosis (mice)		Cardiovascular effects (cats)				
				Dose *mg./kg.*	Effect %	Dose *mg./kg.*	B.P. ± mm. Hg (*min.*)	Resp.	N.M.	Salivation
1.	2-Amino	35	Stimulation and higher doses tonic convulsion	10 20	—40 0	2	+37(15)	0	0	—
2.	3-Amino	28	do	6	—16	2	+15(10)	0	0	0
3.	4-Amino	10	do	2·5	—60	2	+31(20-40)	—	—	—
4.	2,3-Diamino	25	do	5	—56	2	+25(20-30)	—	—	—
5.	3,4-Diamino	20	do	5 15	—42 —64	2	+51(30-50)	++	+++	+
6.	2,5-Diamino	50	do	12·5 25	—14 —40	2	+20(20)	0	+	+
7.	2,6-Diamino	100	Stimulation clonic convulsion	40 20	—41 0	2	+20(15-30)	0	+	+
8.	2,4-Diamino	200	Ataxia	50 100	0 0	2	+10(7)	0	0	0

TABLE 2.—PHARMACOLOGICAL ACTIVITY OF SOME AMINO-IMIDAZO AND TRIAZOLO-PYRIDINES

S. No.	Pyridine derivatives	Approx. LD_{50} (MICE) *mg./kg. i.p.*	General effects (MICE)	Effect on barbiturate hypnosis (MICE)		Cardiovascular effects (cats)		
				Dose *mg./kg.*	Effect %	Dose *mg./kg.*	B.P. (mm. Hg) (max.)	R.
9	3-β-Diethylaminoethylimidazo (4,5-b)	100	Stimulant, at higher dose tonic convulsions	50	—46	3	0	0
10.	3-β-Diethylaminoethyl-6-nitroimidazo (4,5-b)	100	do	50	—41	3	—15(5)	0
11.	3-β-Diethylaminoethyl-6-methoxycarbonylimidazo (4,5-b)	150	do	50	—40	4	+25(10)	+
12.	3-β-Phenylethyl-6-methoxycarbonyl-imidazo (4,5-b)	500*	Respiratory stimulant	..	..	7·5	0	++
13.	3-β-Phenylethyl-6-carboxamido-imidazo (4,5-b)	500*	do	..	..	7·5	—25(2)	+++ (60'-90')
14.	N,N-Diethyl-3-β-phenylethyl-imidazo (4,5-b) pyridine-6-carboxamide	500*	do	..	..	7·5	—40(7)	+++ (60'-90')
15.	4-β-Phenylethylamino-3-amino	150	Depressant anticonvulsant	80	0	5	—72(5)	0
16.	1-β-Phenylethyltriazolo (4,5-c)	400	Depressant	50	+60	2	0	0
17.	1-β-Piperidylethylimidazo (4,5-c)	150	Depressant	50	+50	2	0	0
						3 (dog)	—50(50)	+++ (30')
18.	1-Phenyl-4-(3-amino-4-pyridyl) piperazine	75	Stimulant, anticonvulsant, at higher dose clonic convulsion	25	0	10	60(30)	0

*Oral LD_{50}

The anticonvulsant and depressant activity of 4-β-phenylethylamino-3-aminopyridine (15) and the stimulant activity of 1-(3-amino-4-pyridyl)-4-phenyl piperazine (18) provided clues for further exploration in compounds belonging to these two groups.

1-Pyridyl-4-arylpiperazines

Certain selected variations were introduced in parts A, B and C of the molecule of 1-(3-amino-4-pyridyl)-4-phenylpiperazine (Fig. 2) to study their structure-activity relationship. The methods of synthesis are illustrated in Fig. 3.

These compounds were tested for their gross effects, anticonvulsant, antireserpine and cardiovascular activities. The pharmacological activity for some selected compounds, described in Table 3 shows that:

(i) If the arylpiperazinyl residue is attached to position 4 of the pyridine ring, the compounds possess anticonvulsant and central stimulant activity as shown by their antireserpine action. These activities were most marked

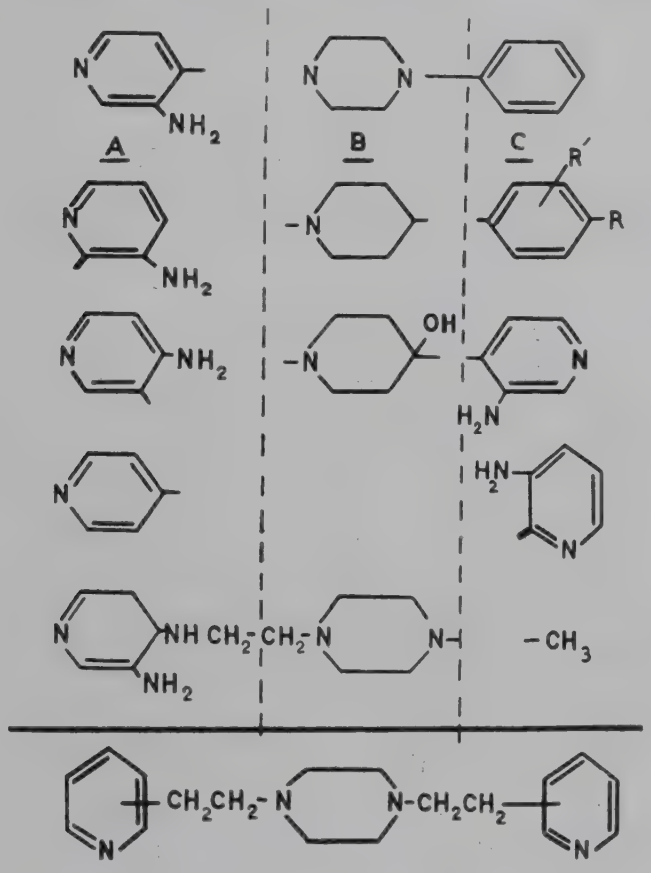


FIG. 2—VARIATIONS MADE IN 1-(3-AMINO-4-PYRIDYL)-4-PHENYLPIPERAZINE

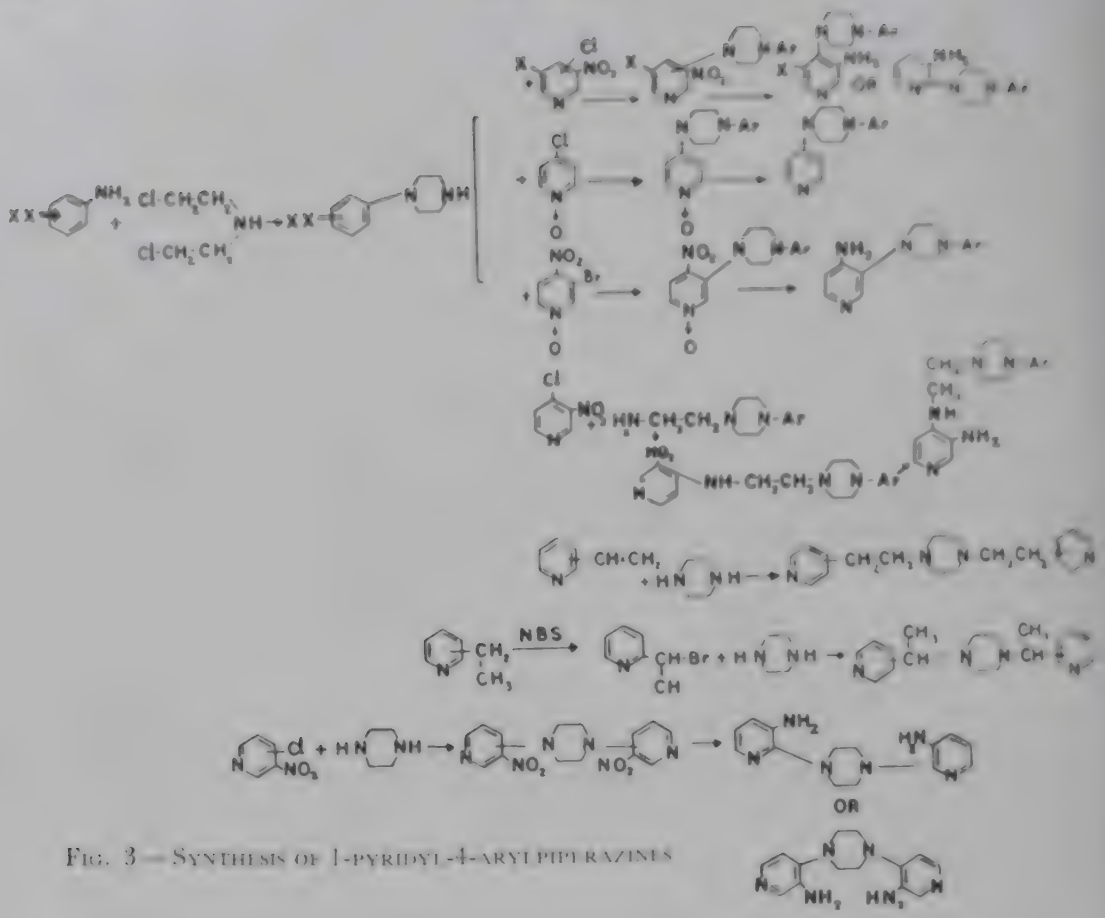


FIG. 3 — SYNTHESIS OF 1-PYRIDYL-4-ARYLPIPERAZINES

in the case of 4-phenyl and 4-(4-chlorophenyl) piperazine (19 and 24); other substituted phenyl piperazines so far tested, had a lower activity.

(ii) The removal of the amino group from the pyridine nucleus reduced antireserpine activity, while retaining the anticonvulsant action as in 1-pyridyl-4-phenyl piperazine (22).

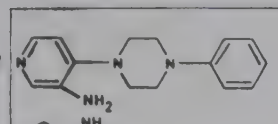
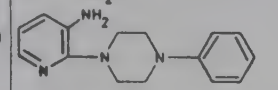
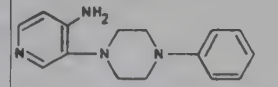
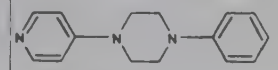
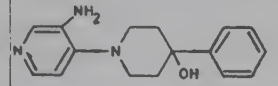
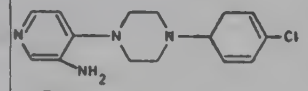
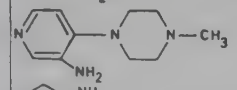
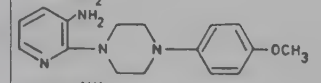
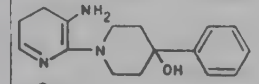
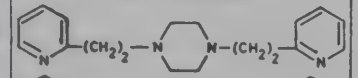
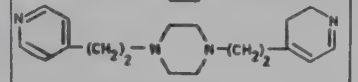
(iii) The replacement of an arylpiperazine by a 4-aryl-4-hydroxypiperidine residue considerably reduced this antireserpine activity while the anticonvulsant activity was more or less unaffected as in 1-(3-amino-4-pyridyl)-4-phenyl-4-hydroxypiperidine (23).

(iv) The aryl ring seems to be essential for both stimulant and anticonvulsant activities, which disappeared when aryl residue was replaced by an alkyl group as in 1-(3-amino-4-pyridyl)-4-methyl piperazine (25) or a heterocyclic group as in 1, 4-bis (3-amino-4-pyridyl) piperazine. Compounds 28 and 29 showed depressant action but lacked any significant anticonvulsant action.

(v) If the arylpiperazine and amino groups were in position 3 and 4 respectively of the pyridine ring (21), the central stimulant and anticonvulsant activities were considerably reduced.

(vi) When the arylpiperazine residue was attached to position 2 of the pyridine ring, the compound (20) showed depressant action in the gross

TABLE 3

	Structure	Approx. LD_{50} (mice) *mg./kg. i.p.*	Gross effects	MES block	Antireserpine (mice)			Cardiovascular activity (cat)			
					Anti-hypothermia	Anti ptosis	Anti sedation	Effect on b.p. at 2·5 mg./kg. i.v.	Adrenaline	ACh	NM
19		100	Stimulant. Antipyretic +, Anti-inflammatory +	5+	4+	4+	4+	−50 (P) 10 mg.	↓	—	—
20		150	Depressant	±	—	±	—	−40 (T)	—	—	
21		50	Stimulant	+	—	±	±	+30 (P)	↑	↓	
22		25	do	4+	+	+ +	±	−30 (P)	↑	—	—
23		50	do	4+	—	±	±	−60 (T)	—	—	↑
24		80	Depressant, Antipyretic + + Anti-inflammatory	5+	4+	4+	4+	—	—	—	
25		>200	Stimulant	—	—	—	—	± 10 (T)	—	—	—
26		>400	Depressant	4+	+	±	—	+40 (T)	↑	0	0
27		100	do	4+	Enhances depression			—	—	—	—
28		80	Depressant, Antiamphetamine, Antipyretic + +	+	—	—	—	—	—	—	—
29		100	Depressant, Antiamphetamine + +	+	—	—	—	—	—	—	—

T, Transient up to 5ft P, Persistent up to 10ft or above −, No effect 0, Not done ↓, Decrease ↑ Increase

behaviour and also did not possess any anticonvulsant action. This depressant action was enhanced by replacing the 4-N of piperazine by a C-OH group [27]. The latter also showed marked anticonvulsant activity and enhanced reserpine induced depression in mice.

ω-Arylalkylamino-aminopyridines

The different types of compounds synthesized in this group and their methods of synthesis are described in Fig. 4. The pharmacological activity of some of the compounds is described in Table 4.

The introduction of substituents like bromo, nitro [31] or amino groups in position 5 of 4-β-phenylethylamino-3-amino-pyridine [30] abolished its anticonvulsant activity. Branching of the alkyl chain [32] or an increase in the chain length markedly reduced the anticonvulsant activity. These compounds showed a transient vasodepressor response. Introduction of 3, 4-dimethoxy [33], 3, 4-dimethyl [39] and 3, 4-dihydroxy [34] groups in the phenyl ring also reduced the anticonvulsant action.

The 4-[β-(3, 4-dihydroxyphenyl) ethylamino]-3-amino-pyridine [34] showed interesting action on the blood pressure. At a dose of 2·5 mg./kg. i.v. in cats, it showed a transient vasopressor action, while at 10 mg./kg. it showed a transient vasopressor action followed by marked vasodepressor response of a rather long duration. In intact and spinal cats it selectively blocked the pressor action of noradrenaline but potentiated the action of adrenaline at a dose of 10 mg./kg. i.v. In dogs at 3 mg./kg. i.v. there was fall in blood pressure of 20 to 30 per cent, which lasted for two and a half hours. Respiratory rate and heart rate were not markedly altered. When given

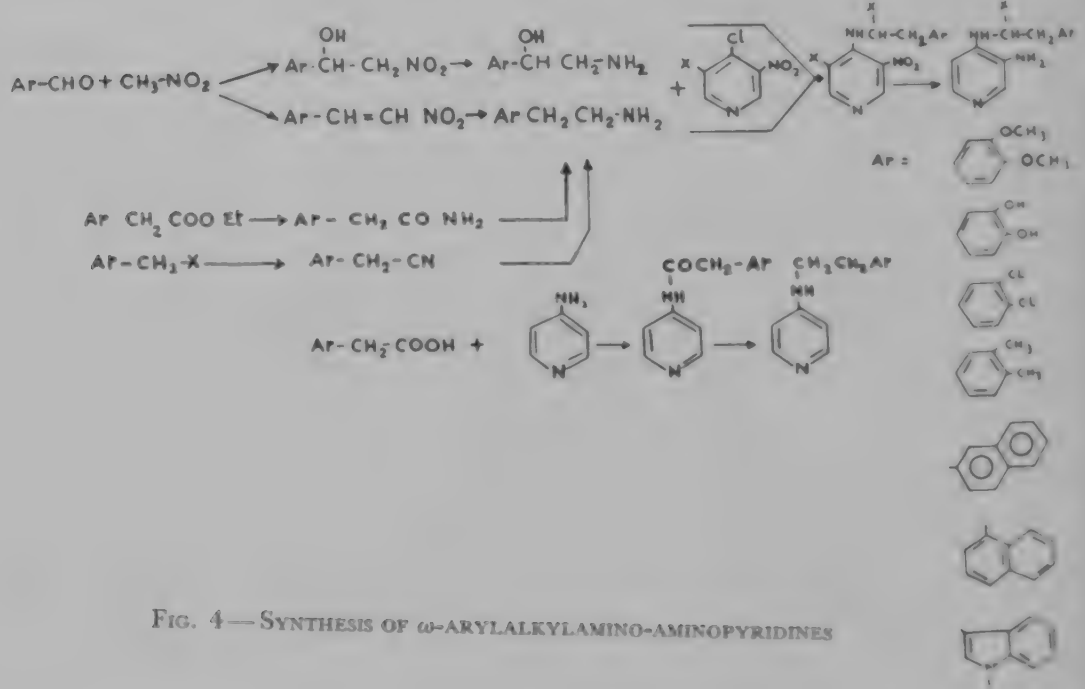
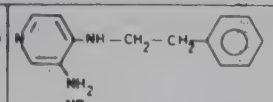
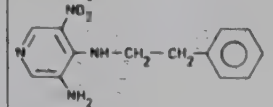
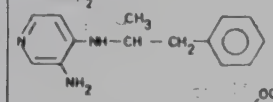
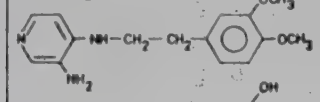
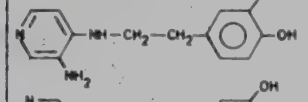
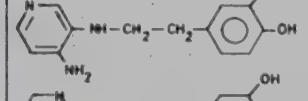
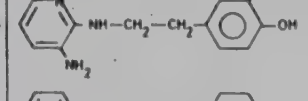
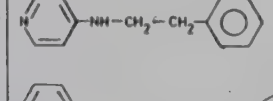
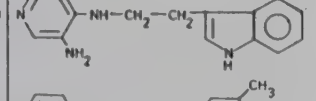
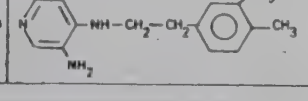


FIG. 4 — SYNTHESIS OF ω-ARYLALKYLAMINO-AMINOPYRIDINES

TABLE 4

	Structure	APR LD_{50} (mice) *mg./kg. i.p.*	Gross effect	Cardiovascular activity (cat)				MES block (mice)
				Dose *mg./kg. i.v.*	Effect on b.p. *mm./Hg.*	ADR	ACh	NM	
30		150	Depressant	5	−72 (T)	↑	—	—	++
31		800	do	2	−40 (T)	—	—	—	—
32		80	do	5	−25 (T)	↑	—	—	+
33		250	do	2·5	−10 (T)	↑	—	—	—
34		150	Mixed	2·5 10	+30 (T) −70 (P)	↑	—	—	—
35		150	do	2·5	+30 (P)	↑	—	↑	—
36		>800	Sedation	3·0 (dog)	—	—	—	0	↓
37		20	Stimulant	2·5	+60 (P)	(15)	—	↑	+++
38		80	Depressant	2·5	—	—	—	—	0
39		>100	Stimulant	—	—	—	—	—	0

↑, Potentiation ↓, Block —, No effect 0, Not done

intraduodenally this compound had no effect on the blood pressure thus indicating that it was not absorbed from the gastrointestinal tract. The corresponding 3-(β-3, 4-dihydroxyphenylethylamino)-4-aminopyridine (35) and 2-(β-3, 4-dihydroxyphenylethylamino)-3-aminopyridine (36) did not show this vasodepressor action. 4-[β-(3-Indolyl) ethylamino]-3-aminopyridine (38) and 4-[β-(1 and 2-naphthyl) ethylamino]-3-aminopyridines also did not show any hypotensive action.

In view of the interesting tranquillizing activity shown by 1-β-phenylethyltriazolo (4, 5-c) pyridine (16) and CNS stimulant and anticonvulsant activities shown by 1-(3-amino-4-pyridyl)-4-phenylpiperazine (18), their pharmacology was studied in detail.

1-β-Phenylethyltriazolo (4, 5-c) pyridine (16)

Depending on dose it produced tranquillization, sedation, catalepsy and loss of righting reflex in mice and rats. Its sedative effects were characterized by reduced spontaneous motor activity and reactivity and increased passivity. Quietening effects were also observed on rabbits and dogs.

It caused moderate hypothermia in mice and rats at 24°C., but like chlorpromazine and reserpine, it failed to produce hypothermia and sedation at an ambient temperature of 37°C.

It significantly potentiated barbiturate and ethanol induced narcosis in mice. Barbiturate and ethanol potentiation was seen with doses which did not produce detectable sedation and hypothermia. It was also effective in inducing sleep at sub-hypnotic dose level of pentobarbitone. Its hypnotic potentiating effect, like chlorpromazine and unlike reserpine, was not altered at 37°C. (Table 5).

The compound showed very weak analgesic effect in mice by itself but enhanced significantly morphine analgesia (hot-plate method[7]. It showed a marked antipyretic action. It decreased amphetamine and methylphenidate induced hyperactivity, but did not antagonize amphetamine toxicity, in aggregated mice. No significant effects on electroshock and chemoshock induced seizures were observed; rather it slightly enhanced pentylenetetrazole induced convulsions and death.

Iproniazid and imipramine pretreatment partially reduced its hypnotic potentiation and nearly abolished that of reserpine but did not alter this effect of chlorpromazine (Table 6). Its hypothermic and sedative effects in mice were significantly reduced by pretreatment with imipramine while in iproniazid treated mice, sedation was counteracted but not the degree of hypothermia though the duration was reduced.

It produced a weak but specific block of the conditioned avoidance response (Table 7) and increased the reaction time to conditioned stimulus (Cook and Weidley[8]). It also potentiated the effect of chlorpromazine and reserpine on conditioned avoidance response.

It was effective in impairing learning (acquisition) and retrieval of

TABLE 5—EFFECT OF COMPOUND (16) ON SLEEPING TIME IN MICE

Drugs	Dose *mg./kg.*	At 24°C. Ethanol (20%) 15 *ml./kg. i.v.* (mean 95% *f.l. min.*)	At 24°C. Pentobarb. sod. 50 *mg./kg. i.p.* (mean 95% *f.l. min.*)	At 37°C. Pentobarb. sod. 50 *mg./kg. i.p.* (mean 95% *f.l. min.*)
Control	(—)	2·7 (2·1-3·4)	50·0 (42-60)	45 (33-61)
Compound (16)	25	4·4 (3·6-5·3)	80·0 (69-93)	(—)
	50	6·7 (5·2-8·7)	101 (84-122)	(—)
	100	11·5 (8·9-13·1)	170 (147-197)	157 (129-191)
Chlorpromazine	3	19·0 (15·1-25·7)	151 (129-177)	132 (107-164)
Reserpine	1·5	7·5 (58-97)	111 (92-130)	+57 (45-74)

(—) Mean potentiation with respect to control was highly significant (RL 0·01)
\+ Not significant

TABLE 6—EFFECT OF COMP. (16) ON SLEEPING TIME IN IMIPRAMINE OR IPRONIAZID PRETREATED MICE

(Eight mice were used in each group)

Drugs	Dose *mg./kg.*	A. Without imipramine (mean 95% *f.l. min.*)	B. With imipramine (mean 95% *f.l. min.*)	P (A—B)	A′. Without iproniazid (mean 95% *f.l. min.*)	B′. With iproniazid (mean 95% *f.l. min.*)	P (A′—B′
Control	..	53 (41-68)	58 (45-72)	N.S.	57 (46-70)	55 (44-69)	N.S.
Compound (16)	50	110 (88-137)	82 (66-102)	0·05	110 (91-133)	63 (51-75)	0·01
	100	164 (128-212)	113 (97-131)	0·05	159 (124-204)	112 (93-136)	0·05
Chlorpromazine	3	160 (121-211)	164 (140-193)	N.S.	144 (113-186)	147 (119-182)	N.S.
Reserpine	1·5	116 (95-141)	84 (71-100)	0·05	112 (98-129)	53 (93-66)	0·01

TABLE 7 -EFFECT OF COMPOUND (16) ON CONDITIONED AVOIDANCE RESPONSE (CAR) IN TRAINED RATS

Drugs	Dose *mg./kg.*	Block of CAR %	ED_{50} (95% fiducial limits) *mg./kg.*
Compound (16)	50	20	++
	75	25	103 (204–156·6)
	100	44·4	
	125	66·6	

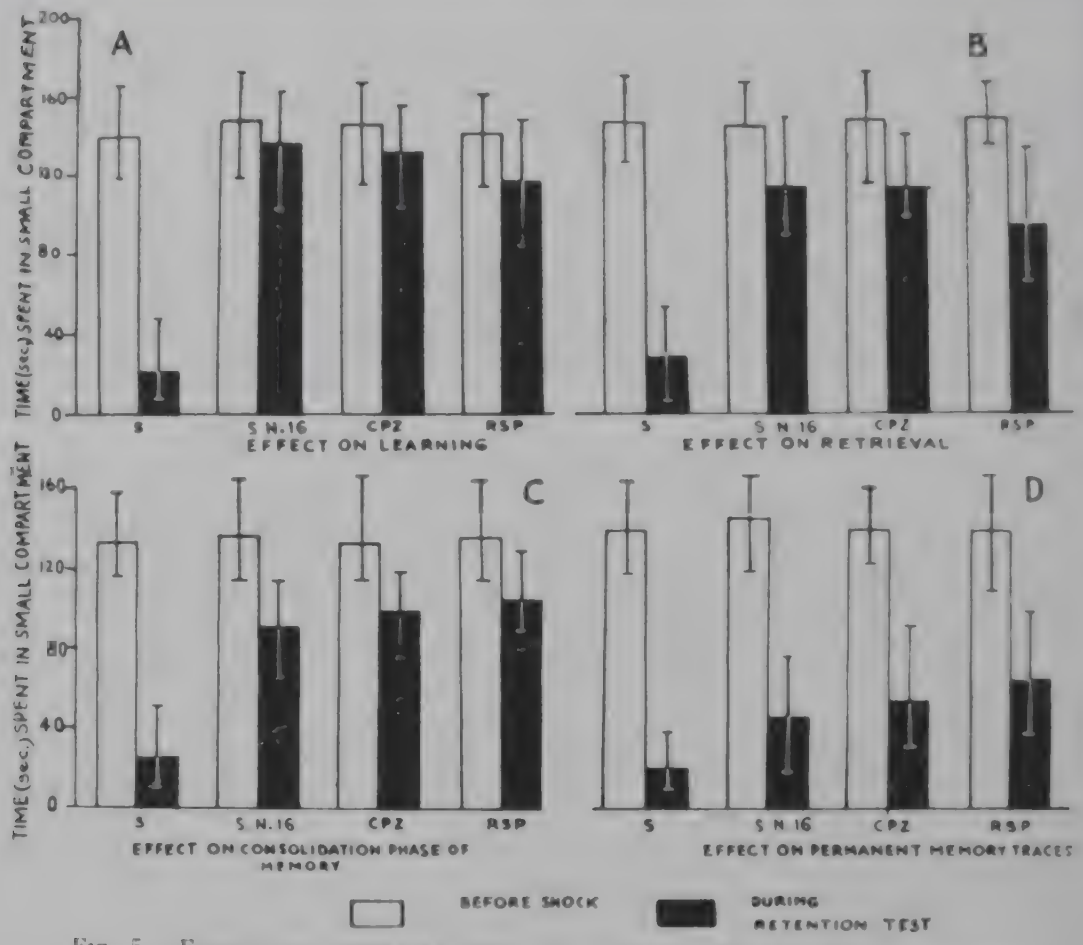


FIG. 5—EFFECT OF DRUGS ON PASSIVE AVOIDANCE RESPONSE (ONE TRIAL LEARNING PROCEDURE). [Columns indicate the average time with range (vertical lines) spent by the rats in the small compartment before shock and during retention test. S. (saline); S. N. 16, 25 mg./kg.; CPZ (chlorpromazine), 1 mg./kg.; RSP. reserpine, 0·5 mg./kg.]

passive avoidance reactions in rats (one trial learning procedure[9]). The compound also impaired consolidation phase of memory trace formation but did not affect the permanent memory (Fig. 5).

EEG recordings, on oscilloscope, from bipolar surface electrodes in the rabbit revealed that it produced high voltage slow wave activity.

It produced a transient fall in blood pressure in anaesthetized cats, negative ionotropic effect on isolated guinea-pig heart and a weak non-specific spasmolytic activity on isolated smooth muscle. No significant effect on the respiration, evoked contraction of nictitating membrane and neuromuscular transmission was found.

The profile of pharmacological activity of compound (16) thus shows that it is a weak tranquillizer which has some actions like those of chlorpromazine and reserpine and some which are unique to the compound.

1-Phenyl-4-(3-amino-4-pyridyl)-piperazine (18)

At low dose the compound did not show any significant effect on the gross behaviour of mice except a little increase in alertness, while at higher dose it produced excitement, jumping and convulsions. Sign of stimulation or alertness was found to be correlated with EEG patterns.

In mice ($\frac{1}{4}$ to $\frac{1}{2}$ LD_{50} dose) it showed marked antiextensor activity against maximal electroshock (MES), pentylene tetrazole and strychnine induced tonic convulsions (Table 8). No protection against the pentylene tetrazole induced clonic convulsions was seen; rather the compound slightly enhanced the frequency and duration of clonic phase. The compound, however, failed to protect the mortality caused by metrazol or strychnine.

Its effect on reserpine induced hypothermia, ptosis and sedation is given in Table 9. The results show that the compound when injected after 4 hr of administration of reserpine (3 mg./kg. i.p.) reversed the fall in body temperature and completely counteracted ptosis and sedation. This effect of the drug lasted for nearly one hour.

Its effects on somatic reflexes were studied[10,11] (Figs. 6 and 7). In doses of 5–10 mg./kg. i.v. it blocked the linguomandibular flexor and crossed

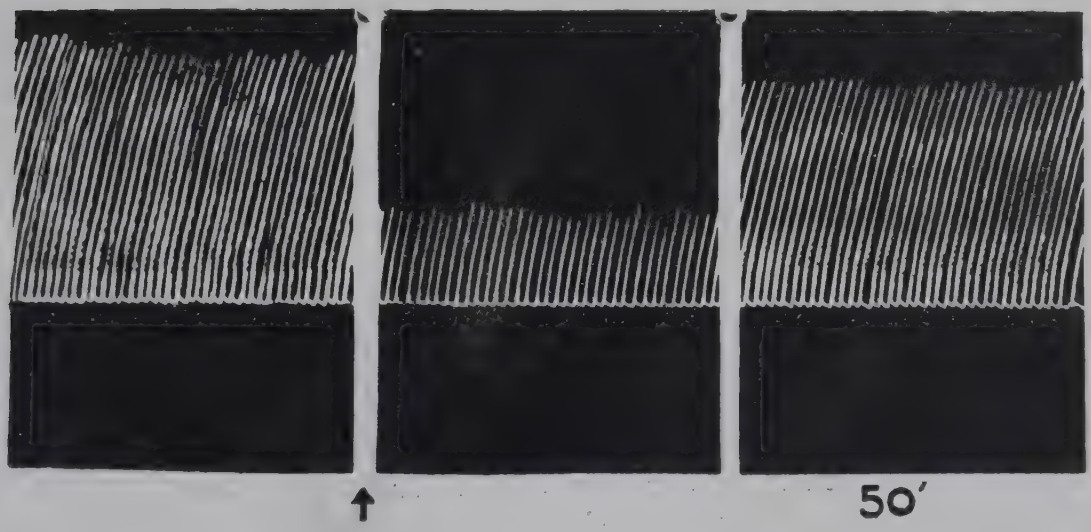


FIG. 6 — EFFECT OF S. No. 18, 5 mg./kg. i.v. (AT ARROW) ON THE LINGUOMANDIBULAR REFLEX IN INTACT CHLORALOZED CAT

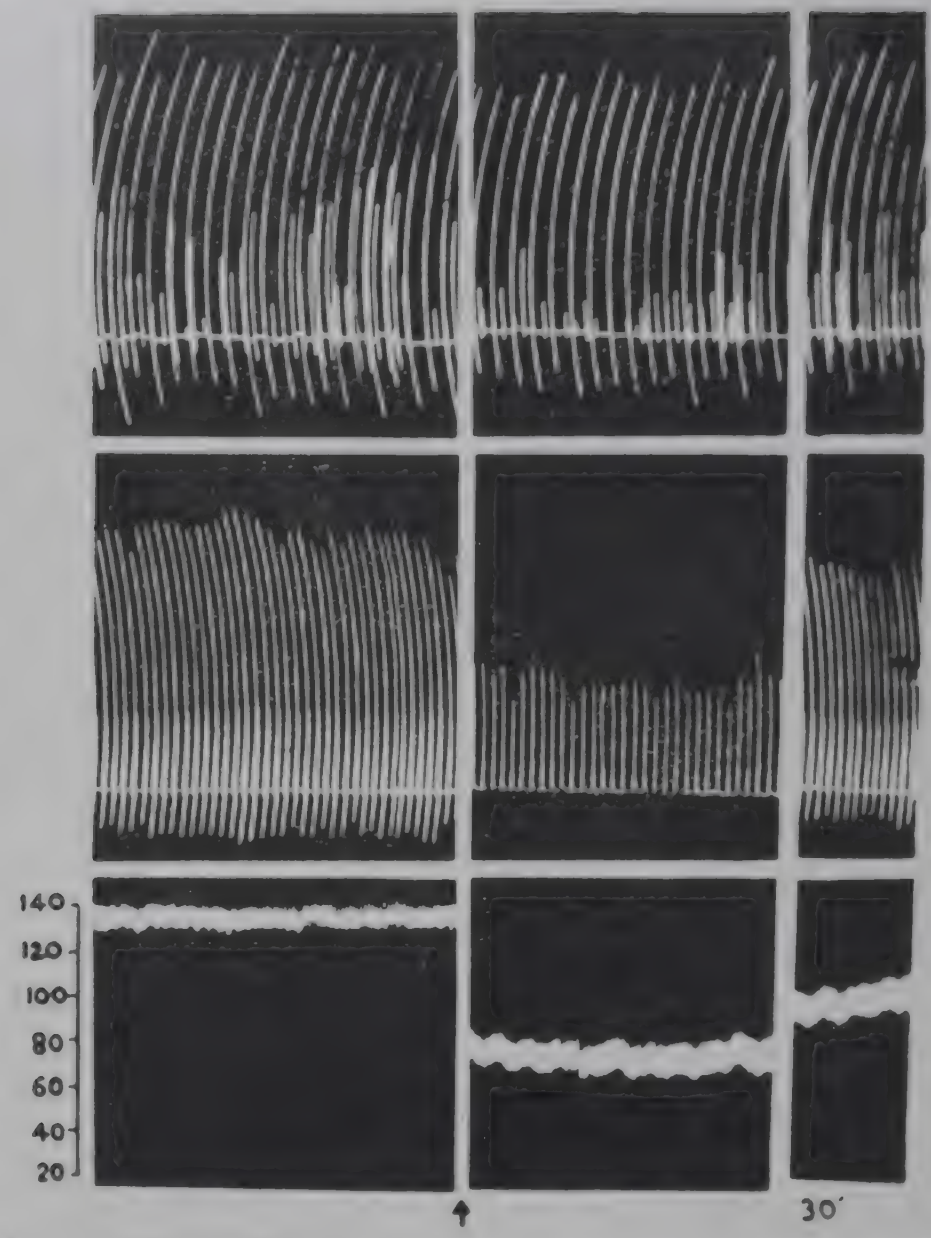


FIG. 7 — EFFECT OF S.N. 18, 5 mg./kg. i.v. (AT ARROW) ON MONO- AND POLYSYNAPTIC REFLEXES IN INTACT CHLORALOSED CAT [Upper tracing, patellar reflex (bigger lines) and crossed extensor reflex (smaller lines); Middle tracings, flexor reflex; Lower tracings, blood pressure]

extensor reflexes (polysynaptic) by 40–60 per cent, but slightly enhanced the patellar reflex (monosynaptic) in some cases. The effect on the response of the flexor reflex was found to be similar in intact and in the spinal cat. Inhibitory effect on polysynaptic reflex lasted for 40–60 min.

In doses of 5–10 mg./kg. i.v. it produced a sustained fall in blood pressure (50–60 mm. Hg. lasting for about 30 min. The compound at 10 mg./kg. i.v., caused slight block of adrenaline and marked block of noradrenaline response on blood pressure. The effect of histamine, acetylcholine

TABLE 8—EFFECT OF COMPOUND (18) (25 mg./kg.) ON EXPERIMENTALLY INDUCED CONVULSIONS IN MICE

Convulsants	% Protection against	
	Tonic con.	Death
MES (48 MA, 0·2 sec.)	100·0	..
Metrazol (90 mg./kg. s.c.)	100·0	0·0
Strychnine (1·5 mg./kg. i.p.)	100·0	0·0*

*Survival time was increased (twice as compared to control)

TABLE 9—ANTIRESERPINE EFFECT OF COMPOUND (18)

Drug	Reserpine, 3 *mg./kg. i.p.*		
	Mean fall in body temp.	Ptosis	Sedation
Control	9·4°F.	++++	+++
Compound (18) (25 mg./kg. i.p.)	2·0°F.	0	0

0, absent

or stimulation of the peripheral end of the cut vagus nerve on blood pressure remained unaltered. The compound did not alter the response of the nictitating membrane contraction to the stimulation of preganglionic fibres of the superior cervical ganglion. No effect was observed on the neuromuscular transmission.

All the known anticonvulsants carry in their molecules an amido function. Thus, compound 18, which lacks an oxygen function, is a new structural type possessing anticonvulsant activity. Its significant antireserpine activity adds further interest to this class of compounds.

REFERENCES

1. Jain, P. C., Chatterjee, S.K. & Anand, N., *Indian J. Chem.*, **1** (1963), 30, and earlier papers.
2. Vohra, M. M., Pradhan, S. N., Jain, P. C., Chatterjee, S. K. & Anand, N., *J. med. Chem.*, **8** (1965), 296.
3. Fastier, F. N., *Pharmac. Rev.*, **14** (1962), 37.
4. Hexathausen, E. V., *ibid.*
5. Vohra, M. M. & Pradhan, S. N., *Arch. int. Pharmacodyn.*, **150** (1964), 413.
6. Hunger, A., Kebrle, J., Rossi, A. & Hoffman, K. N., U.S. Patent 3,044,978 (Oct. 17, 1961); *Chem. Abstr.*, **56** (1962), 4771.
7. Eddy, N. B. & Leimbach, D., *J. Pharmac. exp. Ther.*, **107** (1953), 385.
8. Cook, L. & Weidley, E., *Ann. N.Y. Acad. Sci.*, **66** (1957), 740.
9. Buresova, O., Bohdanecky, Z., Bures, J. & Weiss, T., *Int. Pharmac. Meeting*, **1** (1963), 351 (ed. Mikhelson, Longo and Votava) Pergamon Press.
10. King, E. E. & Unna, K. R., *J. Pharmac. exp. Ther.*, **111** (1954), 293.
11. Witkin, L. B., Spitaletta, P. & Plummer, A. J., *Arch. int. Pharmacodyn.*, **124** (1960), 105.

Participation of an Unusual Ganglionic Pathway in the Mediation of the Pressor Effect of Physostigmine in Rat

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The pressor response to physostigmine in the rat arises from an activation of central nervous sympathetic mechanism (Varagic, 1955; Lesic and Varagic, 1961; Varagic and Vojvodic, 1962). It is usually assumed that this response is mediated by way of the established peripheral sympathetic pathways (Varagic, 1965; Dirnhuber and Cullumbine, 1955). In an earlier paper (Gokhale, Gulati & Joshi, 1963) it was reported that hexamethonium, a highly selective ganglion-blocking agent but used in doses (100 mg./kg.) for above those usually effective in blocking autonomic ganglia and which completely blocked the pressor responses to dimethylphenylpiperazinium, only partially blocked the pressor effect of physostigmine; this result suggested that the central sympathetic excitation initiated by physostigmine may be partly mediated by a ganglionic pathway insensitive both to block by hexamethonium and to stimulation by dimethylphenylpiperazinium. On the other hand, it is also possible that in the presence of hexamethonium physostigmine raises blood pressure through a direct excitation of ganglionic receptor sites which are pharmacologically different from those usually associated with transmission through sympathetic ganglia. A similar mechanism has been suggested for the pressor effect of physostigmine in dogs after block of ganglia by hexamethonium (Hilton, 1961).

This paper describes experiments which were designed to examine these possibilities.

METHODS

Albino rats of either sex weighing between 200 and 300 g. were anaesthetized with urethane (1·5 g./kg. intraperitoneally, in a 20% w./v. solution). Arterial blood pressure was recorded from a polyethylene cannula tied into a common carotid artery and connected to a Sanborn electromanometer (Model 121C) and a Sanborn Twin-Viso recorder (Model 60-1300). Injections were made through a polyethylene cannula in an external jugular vein. Drugs were injected in a constant volume of 0·1ml., and washed in by the same volume of 0·9 per cent saline. Spinal rats were prepared by dividing the spinal cord at the level of the second cervical vertebra and destroying the brain by pushing a metal probe through the foramen magnum. Ether anaesthesia was used in the preparation of spinal rats. Artificial ventilation was given by means of a miniature 'Ideal' pump (Palmer).

Drugs. Physostigmine salicylate, hexamethonium chloride, dimethyl phenylpiperazinium iodide, atropine sulphate, cocaine hydrochloride, (±)-noradrenaline hydrochloride, *N*-diethylaminoethyl-*N*-isopentyl-*N'*, *N'* di-isopropylurea (P-286), 4-(*m*-chlorophenylcarbamoyloxy)-2-butynyltrimethylammonium chloride (McN-A-343) and 3-acetoxy-1-benzyl-1-methylpyrrolidinium bromide (AHR 602) were used; the doses refer to the salts. Adrenaline base and nicotine base were dissolved in 0·9 per cent saline immediately before injection and the doses refer to the bases. Heparin (1,000 μg./kg.) was injected intravenously as an anticoagulant.

DESIGN OF EXPERIMENTS

Pressor responses to adrenaline or noradrenaline were used to test vascular reactivity. Dimethylphenylpiperazinium was used as a classical or nicotinic ganglion stimulant (Levy and Ahlquist, 1962). McN-A-343 and AHR 602 were used as unusual or non-nicotinic ganglion stimulants (Roszkowski, 1961; Franko, and Alphin, 1963). Hexamethonium was used as a conventional ganglion-blocking agent. Atropine and cocaine were used as drugs which selectively block the action of the non-nicotinic ganglion stimulating agents (Roszokowski, 1961; Jones, Gomez Alonso de la Sierra and Trendelenburg, 1963). P-286 and nicotine antagonize ganglion stimulants of both categories (Levy and Ahlquist, 1962; Jones *et al.*, 1963), and were used in this capacity.

A fixed dose (60 μg./kg.) of physostigmine, injected intravenously at 30–40 min. interval, produced a rise of blood pressure, which was remarkably constant in magnitude for a period of 5–6 hr during individual control experiments, although the value varied from rat to rat (50–75 mm./Hg). After two equipressor responses to physostigmine had been obtained, the following 'test drugs' were injected at 15 min. intervals: noradrenaline (1–1·5 μg./kg.), dimethylphenylpiperazinium (60–100 μg./kg.), McN-A-343 (100–200 μg./kg.) and AHR 602 (300 μg./kg.). This sequence was

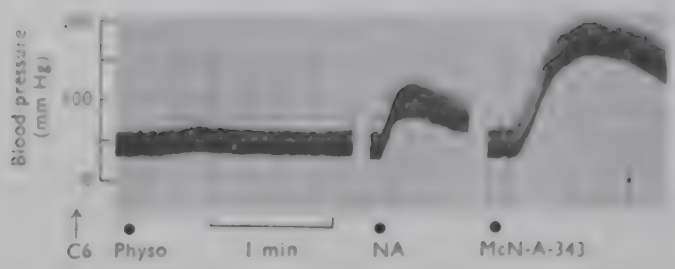

FIG. 1—SPINAL RAT, 260 g. ARTIFICIAL VENTILATION RECORD OF CAROTID ARTERIAL BLOOD PRESSURE [Responses to physostigmine (120 μg./kg. at Physo), to noradrenaline (1μg./kg. at NA) and to McN-A-343 (150μg./kg. at McN-A-343). Hexamethonium (C6, 15 mg./kg. in three divided doses) was injected 10 min. before physostigmine was given. Time mark, 1 min. Injections intravenous]

followed by the administration of the blocking drugs; after a specified interval, the responses to physostigmine and the 'test drugs' were again determined.

RESULTS

Spinal Rats

In each of five spinal rats, physostigmine (60 μg./kg.), administered intravenously 40 min. after cutting the spinal cord, did not increase blood pressure. However, responses to all the 'test drugs' were intact. In six other experiments hexomethonium (10–15 mg./kg., in divided doses) was administered 40 min. after cutting the spinal cord, and physostigmine, even in doses two to three times the usual effective dose, then failed to increase blood pressure. In these animals noradrenaline, McN-A-343 and AHR 602 all produced large pressor effects (Fig 1).

Effects of Combined Treatments with Hexamethonium and Atropine and with Hexamethonium and Cocaine

In recent years, investigations of the unusual ganglion stimulant properties of McN-A-343 and AHR 602 have shown that these drugs discharge ganglionic receptor sites which are pharmacologically different from those blocked by hexamethonium, and that their action is specifically blocked by atropine and cocaine (Roszkowski, 1961; Franko and Alpine, 1963; Jones, 1963). The effects of combinations of hexamethonium and atropine and of hexamethonium and cocaine on the pressor response to physostigmine were studied to determine whether these 'muscarinic' ganglionic receptors participate in the mediation of this response.

Hexamethonium and atropine. In nine experiments hexamethonium (20 mg./kg.) produced a small (15–20 mm. Hg) but sustained fall of blood pressure; 15 min. later the pressor effect of physostigmine had been reduced by about half. Simultaneously the pressor effects of noradrenaline, McN-A-343 and AHR 602 were considerably potentiated, whereas the response to dimethylphenylpiperazinium was completely abolished. A small dose (20 μg./kg.) of atropine was administered at this stage, and

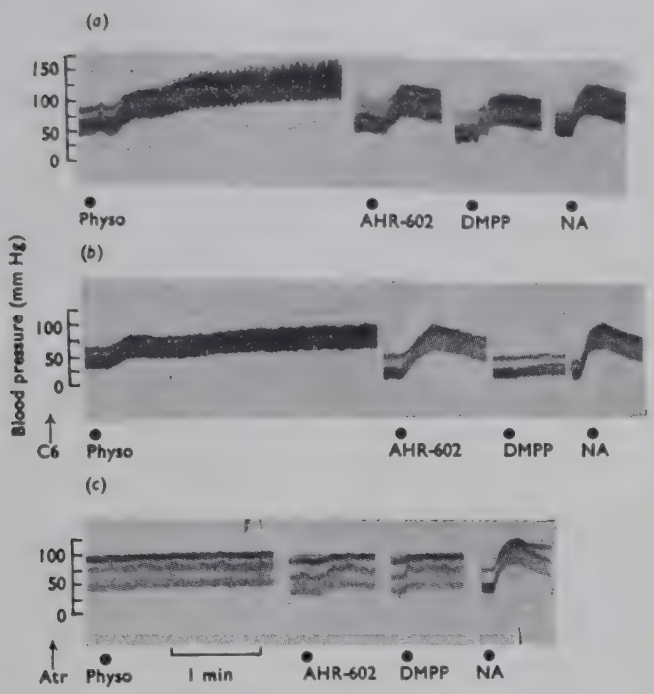

FIG. 2—RAT, 243 g. RECORD OF CAROTID ARTERIAL BLOOD PRESSURE [Responses to physostigmine (60 μg./kg. at Physo), to AHR-602 (300 μg./kg. at AHR-602), to dimethylphenylpiperazinium (60 μg./kg. at DMPP) and to noradrenaline (1·5 μg./kg. at NA). Between (*a*) and (*b*), 20 mg./kg. of hexamethonium (C6) were injected 15 min. before (*b*); between (*b*) and (*c*), 20 μg./kg. of atropine (Atr) were injected 15 min. before (*c*). Time mark, 1 min. Injections intravenous]

15 min. later the pressor responses to physostigmine, McN-A-343 and AHR 602 were totally blocked, but the response to noradrenaline remained (Fig. 2).

In six other experiments atropine (20–30 μg./kg.) by itself produced only a small reduction of the pressor effect of physostigmine but totally blocked the effects of McN-A-343 and AHR 602. Subsequent administration of hexamethonium (20 mg./kg.) in the same animal, however, completely blocked the pressor response to physostigmine.

Hexamethonium and cocaine. In six experiments, cocaine (2–4 mg./kg.) was injected intravenously 10 min. after hexamethonium (20 mg./kg.) had partially blocked the pressor effects of physostigmine; 10–15 min. later physostigmine had no pressor action. The pressor responses to McN-A-343 and AHR 602 were also totally blocked, whereas the pressor effect of noradrenaline remained potentiated.

Cocaine (2–4 mg./kg.) by itself had a rather variable effect on the pressor response to physostigmine but invariably blocked the effects of McN-A-343 in each of the three rats and of AHR 602 in each of three. Out of six experiments only in three was the pressor effect of physostigmine reduced after the administration of cocaine; in the remaining three, cocaine either slightly potentiated (in two) or had no effect (in one) on the response.

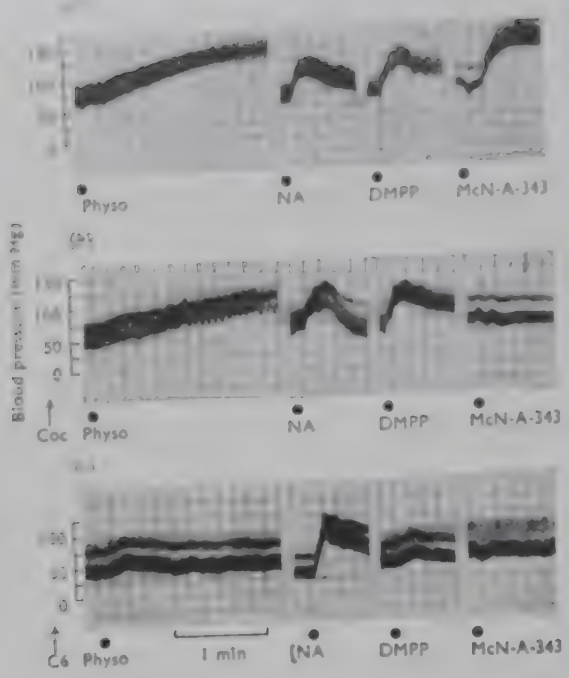

FIG. 3 — RAT, 288 g. RECORD OF CAROTID ARTERIAL B.P. [Responses to: physostigmine (60 μg./kg. at Physo), noradrenaline (1 μg./kg. at NA), dimethylphenylpiperazinium (80 μg./kg. at DMPP), McN-A-343 (100 μg./kg.); between (*a*) & (*b*) 2 mg./kg. cocaine injected 10 min. before (*b*); between (*b*) & (*c*) 20 mg./kg. hexamethonium (C6) injected 11 min. before (*c*). Time mark, 1 min. Injections intravenous]

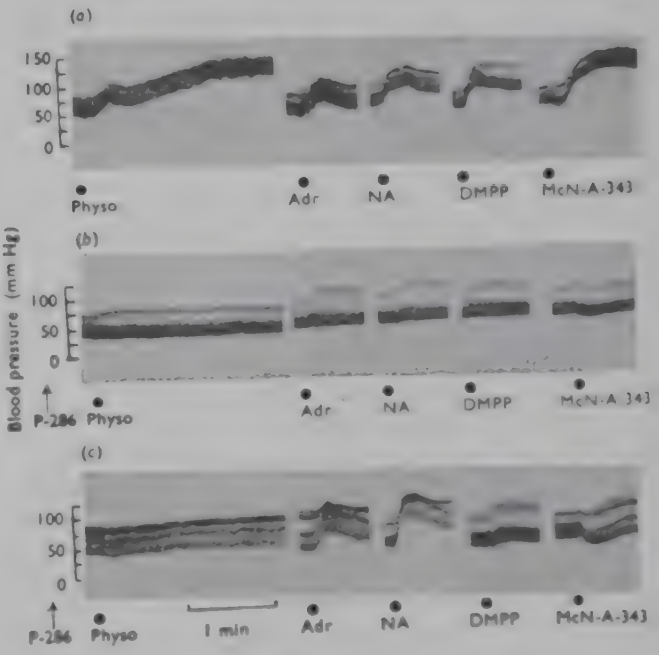

FIG. 4 — RAT, 265 g. RECORD OF CAROTID ARTERIAL B.P. [Responses to: physostigmine (60 μg./kg. Physo), adrenaline (1 μg./kg. Adr), noradrenaline (1 μg./kg. NA), dimethylphenylpiperazinium (80 μg./kg. DMPP), McN-A. 343 (150 μg./kg.); between (*a*) & (*b*), 6 mg./kg. P-286 injected 10 min. before (*b*). Responses in (*c*) obtained 1·5 hr after cumulative admin of 15 mg./kg. P-286. Time mark, 1 min. Injections intravenous]

Subsequent administration of hexamethonium in the same animal, however, always completely abolished the pressor effect of physostigmine (Fig. 3).

Effect of P-286

P-286 (5–10 mg./kg. given slowly intravenously during 5 min.) produced a fall (20–40 mm. Hg) of arterial blood pressure. The drug also induced a conspicuous bradycardia; in twelve experiments the heart rate was reduced by an average of 56 per cent; 10–15 min. after the administration of P-286 the pressor effect of physostigmine as well as responses to all the "test drugs"

were completely abolished (Fig. 4). Over the next 40 min. the blood pressor effects of physostigmine and the 'test drugs' were now completely restored.

At 1–1·5 hr after the cumulative administration of 15–20 mg./kg. of P-286, the blood pressure had fallen to 10–20 mm./Hg, but there was no bradycardia (eight experiments). Now the pressor responses to physostigmine, dimethylphenylpiperazinium, McN-A-343 and AHR 602 were totally blocked but pressor effects of adrenaline and noradrenaline were potentiated (Fig. 4).

Effect of Nicotine

Increasing doses (0·2, 0·4, 0·6 and 1 mg./kg.: 1·2–2·2 mg./kg. total dose) of nicotine were injected intravenously in rapid succession until the drug no longer produced a pressor effect; immediately afterwards the pressor responses to physostigmine, McN-A-343, AHR 602 and dimethylphenylpiperazinium were totally blocked (six experiments). The pressor response to noradrenaline was potentiated (Fig .5).

DISCUSSION

In spinal rats, physostigmine failed to produce a pressor response even after treatment of the animals with hexamethonium, whereas noradrenaline, McN-A-343 and AHR 602 all produced large pressor effects. This observation indicated that the pressor effect of physostigmine is due exclusively to a central stimulant action and also excluded the possibility of a peripheral action of physostigmine accounting for the failure of hexamethonium to block completely the response to physostigmine.

Soon after the administration of P-286 in a dose of 5-10 mg./ kg., the pressor effects of physostigmine, McN-A-343, AHR 602 and dimethylphenylpiperazinium were totally blocked. Pressor responses to adrenaline and noradrenaline were also simultaneously abolished. This result was

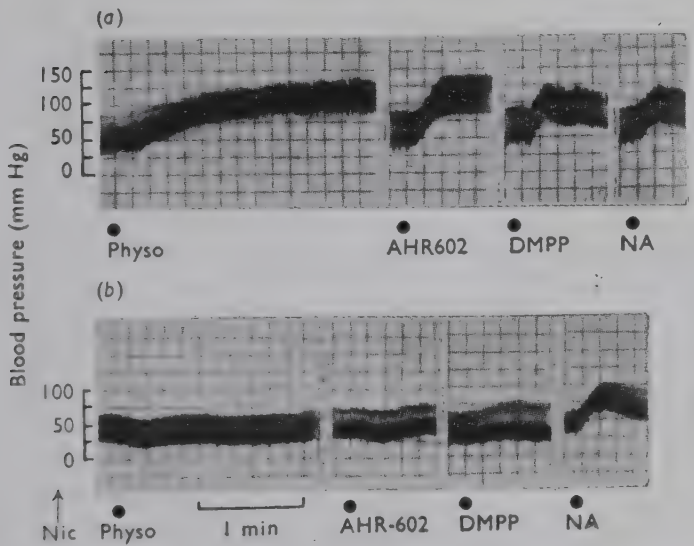

FIG. 5—RAT, 220 g. RECORD OF CAROTID ARTERIAL B. P. [Responses to: physostigmine (60 μg./kg. at Physo), AHR-602 (300 μg./kg. at AHR-602), dimethylphenylpiperazinium (80 μg./kg. at DMPP), noradrenaline (1μg./kg. at NA); between (*a*) & (*b*), nicotine (0·2, 0·4, 0·6 mg./kg.) injected at 2 min. intervals; last injection, which failed to produce a pressor effect, given 2 min. before (*b*). Time mark, 1 min. Injections i.v.]

somewhat unexpected in view of the finding of Levy and Ahlquist (1962) that the pressor and intestinal-inhibitory effects of adrenaline and phenylephrine were not reduced by P-286 (15 mg./kg.). Gardier, Abreu, Richards and Hendlich (1960) also reported that P-286 had no adrenergic blocking action on the dog blood pressure and nictitating membrane. Nevertheless, in the present experiments P-286 (5–10 mg./kg.) invariably blocked the pressor effects of adrenaline and noradrenaline and this could adequately account for the abolition of the pressor effect of physostigmine by P-286 in these conditions, especially as the time course of the two effects was very similar.

Though originally described as a blocking agent specific for the adrenal medulla (Gardier *et al.*, 1960), there is enough evidence in the recent literature to show that P-286 in sufficient doses (15–20 mg./kg.) acts as a classical ganglion-blocking agent with ganglionic effects rather hard to distinguish from those of hexamethonium. Thus P-286 blocks blood pressure responses to dimethylphenylpiperazinium, peripheral vagal stimulation and bilateral carotid artery occlusion (Levy and Ahlquist, 1962; McLean, 1962). In addition, P-286 has an atropine-like action at sympathetic ganglia (Levy and Ahlquist, 1962). In the present experiments, P-286 (1·5–2 hr after administration; 15–20 mg./kg. cumulative dose) produced a sustained fall of blood pressure, abolished the pressor effects of physostigmine, dimethylphenylpiperazinium, McN-A-343 and AHR 602, but enhanced the pressor responses to adrenaline and noradrenaline. Complete block of the pressor response to physostigmine by P-286, which contrasts with the ineffectiveness of even large doses of hexamethonium, could clearly be due to a dual action of the agent on sympathetic ganglia in blocking the effects of both the nicotinic and non-nicotinic ganglion stimulating agents.

Paton and Perry (1953) found that the ganglion block caused by nicotine is biphasic, the initial block by 'depolarization' being followed by a later phase of 'non-depolarizing block' which is similar to the ganglion block produced by hexamethonium. In our experiments, nicotine, during the early depolarizing phase of its blocking action, completely abolished the pressor effects of dimethylphenylpiperazinium as well as those of McN-A-343 and AHR 602 and at the same time totally blocked the pressor effect of physostigmine. Combined treatment with hexamethonium and atropine or with hexamethonium and cocaine also resulted in a total block of the pressor response to physostigmine.

From the evidence presented, it would be reasonable to infer that during block of the established ganglionic pathways (as indicated by a complete abolition of the pressor response to dimethylphenylpiperazinium) the pressor effect of physostigmine is mediated by way of an unusual ganglionic pathway resistant to block by hexamethonium but sensitive to block by atropine, cocaine, P-286 or nicotine. In fact there is good electrophysiological evidence for the existence of functionally distinct neural pathways

through the cat superior cervical ganglion (Bishop and Heinbecker, 1932; Eccles, 1935).

Moreover, in recent years, the results of a number of studies have indicated that pharmacologically distinctive cholinoceptive sites are present in sympathetic ganglia (Ambache, Perry and Robertson, 1956; Eccles and Libet, 1961; Roszkowski, 1961; Takeshige and Volle, 1962, 1963). Thus in addition to the well established nicotinic receptors, atropine-sensitive muscarinic ganglion receptors appear to be fairly strongly established (Jones, 1963). The present results accord well with this concept of pharmacological heterogeneity of ganglionic cholinoreceptive sites and indicate that the muscarinic ganglion receptors might subserve transmission in the suggested unusual ganglionic pathways.

Both atropine and cocaine (in some experiments) by themselves produced a small but appreciable inhibition of the pressor effect of physostigmine. This observation indicates that the muscarinic ganglion receptors might even ordinarily participate in the mediation of this response. This proposal is consistent with the observations of Takeshige and Volle (1962, 1963) that physostigmine unmasks the 'late' component of the post-ganglionic discharge evoked by acetylcholine and that this late response is blocked by atropine.

REFERENCES

1. Ambache, N., Perry, W. L. M. & Robertson, P. A., *Br. J. Pharmac.*, **11** (1956), 442.
2. Bishop, G. M. & Heinbecker, P., *Am. J. Physiol.*, **100** (1932), 519.
3. Dirnhuber, P. & Cullumbine, H., *Br. J. Pharmac.*, **10** (1955), 12.
4. Eccles, J. C., *J. Physiol.* (Lond.), **85** (1935), 179.
5. Eccles, R. M. & Libet, B. *J. Physiol.* (Lond.), **157** (1961), 484.
6. Franko, B. V. & Alphin, R. S., *J. Pharmac. exp. Ther.*, **139** (1963), 25.
7. Gardier, R. W., Abreu, B. E., Richards, A. B. & Herrlich, H. C., *J. Pharmac. exp. Ther.*, **130** (1960), 340.
8. Gokhale, S. D., Gulati, O. D. & Joshi, N.Y., *Br. J. Pharmac.*, **21** (1963), 273.
9. Hilton, J. G., *J. Pharmac. exp. Ther.*, **132** (1961), 23.
10. Jones, A., *J. Pharmac. exp. Ther.*, **141** (1963), 195.
11. Jones, A., Gomez, Alonso de la Sierra, B. & Trendelenburg, U., *J. Pharmac. exp. Ther.*, **139** (1963), 312.
12. Lesic, R. & Varagic, V., *Br. J. Pharmac.*, **16** (1961), 99.
13. Levy, B. & Ahlquist, R. P., *J. Pharmac. exp. Ther.*, **137** (1962), 219.
14. McLean, R. A., Pitman-Moore Research Laboratories (1962).
15. Paton, W. D. M. & Perry, W. L. M., *J. Physiol* (Lond.), **119** (1953), 43.
16. Roszkowski, A. P., *J. Pharmac. exp. Ther.*, **132** (1961), 156.
17. Takeshige, C. & Volle, R. L., *J. Pharmac. exp. Ther.*, **138** (1962), 66.
18. Takeshige, C. & Volle, R. L., *J. Pharmac. exp. Ther.*, **141** (1963), 206.
19. Varagic, V., *Br. J. Pharmac.*, **10** (1955), 349.
20. Varagic, V. & Vojvodic, N., *Br. J. Pharmac.*, **19** (1962), 451.

Neuropharmacological Studies with Imipramine

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Imipramine is chemically related to chlorpromazine and has several properties in common with chlorpromazine. However, imipramine antagonizes the reserpine induced sedation (Sulser *et al.*, 1962) and potentiates the effects of catecholamines (Osborne and Sigg, 1960). The antidepressant properties of imipramine were discovered after prolonged use in patients suffering from endogenous depression (Kuhn, 1957, 1958). Neuropharmacological studies with imipramine have not provided an unequivocal explanation for the antidepressant action of imipramine. The present paper describes the results of a study on the electrocortical arousal induced by mid-brain reticular stimulation in *cerveau isole* cats, the excitability of the cortical neurones in the isolated cerebral cortex (dogs) and the central integration of somatic reflexes in intact and spinal cats.

MATERIALS AND METHODS

In the present investigation fifteen *cerveau isole* cats were successfully employed for mid-brain reticular arousal studies. The details of the technique have already been described in an earlier communication (Saxena *et al.*, 1964). Twenty-five adult mongrel dogs of either sex were used for the isolated cerebral cortex preparation. The neuronal isolation of the cerebral cortex was done by the method of Kristiansen and Courtois (1949) as employed earlier by Sinha *et al.* (1966).

Twenty adult cats were used in this study. The patellar monosynaptic reflex (PR) was elicited by the technique of Calma and Wright (1947). The patellar reflex was subjected to various modifications by the stimulation of facilitatory and inhibitory areas of the reticular formation. The details of the techniques were described in our earlier communications (Bhargava and Srivastava, 1964, 1965a,b). The polysynaptic linguomandibular reflex described by King and Unna (1954) was modified (Bhargava and Srivastava, 1965 b) and employed in the present study.

RESULTS

Electroencephalographic Studies

Effect of imipramine was studied on the electrocortical arousal responses due to mid-brain reticular stimulation in *cerveau isole* preparations. Imipramine (0·1–4·0 mg./kg. i.v.) increased neuronal synchronization and the rate of spontaneous spindles. The threshold of the electrocortical arousal was raised and the duration decreased. The effect of imipramine appeared within 5–7 min., reached its peak in 15 min. and lasted for 4 hr.

Effect of imipramine in doses ranging from 0·1–8·0 mg./kg. i.v. was studied on the spontaneous activity of the intact and the isolated cerebral cortex and on the evoked activity of the isolated cerebral cortex. Imipramine initially induced high voltage slow wave activity both in the intact and isolated cerebral cortex. At the same time the threshold of the after-discharge was raised and the duration decreased. The initial depressant action of imipramine appeared within 5–7 min., reached its peak in 15–20 min. and lasted for six hours. At 375 min., it exhibited a 'stimulant' effect characterized by a desynchronized pattern in the intact cortex, lowering of the threshold and increased duration of the after-discharge in the isolated cerebral cortex. Complete recovery from the stimulant effect was not seen up to 420 min.

The fact, that imipramine exerted a delayed stimulant effect on the neuronal excitability of the cortex, prompted the possibility that a metabolite of imipramine, possibly desmethylimipramine (DMI) might be responsible for the delayed stimulant effect of the drug. Thus it was thought worthwhile to study DMI under comparable experimental conditions.

Effect of DMI (2·0–8·0 mg./kg. i.v.) was studied on the electrocortical arousal responses due to mid-brain reticular stimulation. In contrast with imipramine, DMI decreased the neuronal synchronization and the rate of spontaneous spindles. In the isolated cortical studies, DMI induced high voltage slow wave activity both in the intact and the isolated cortices within 15 min. of drug administration, while the threshold as well as the duration of the after-discharge of the isolated cortex remained unaffected. However, a clear-cut stimulant effect with DMI could be observed at 90 min. after the drug administration. The stimulant effect lasted for 200–250 min. depending upon the dose.

Fig. 1 shows the effects of DMI (8·0 mg./kg. i.v.) on the spontaneous activity of both intact and isolated cerebral cortices and on the threshold of the evoked after-discharge of the isolated cerebral cortex. At 15 min. it induced high voltage slow wave activity in both the cortices but the threshold of the after-discharge was not changed. However, at 90 min. after the drug administration, DMI exhibited a stimulant effect characterized by low voltage fast wave activity in the intact cortex and fall in the threshold of the after-discharge in the isolated cerebral cortex. Though the threshold

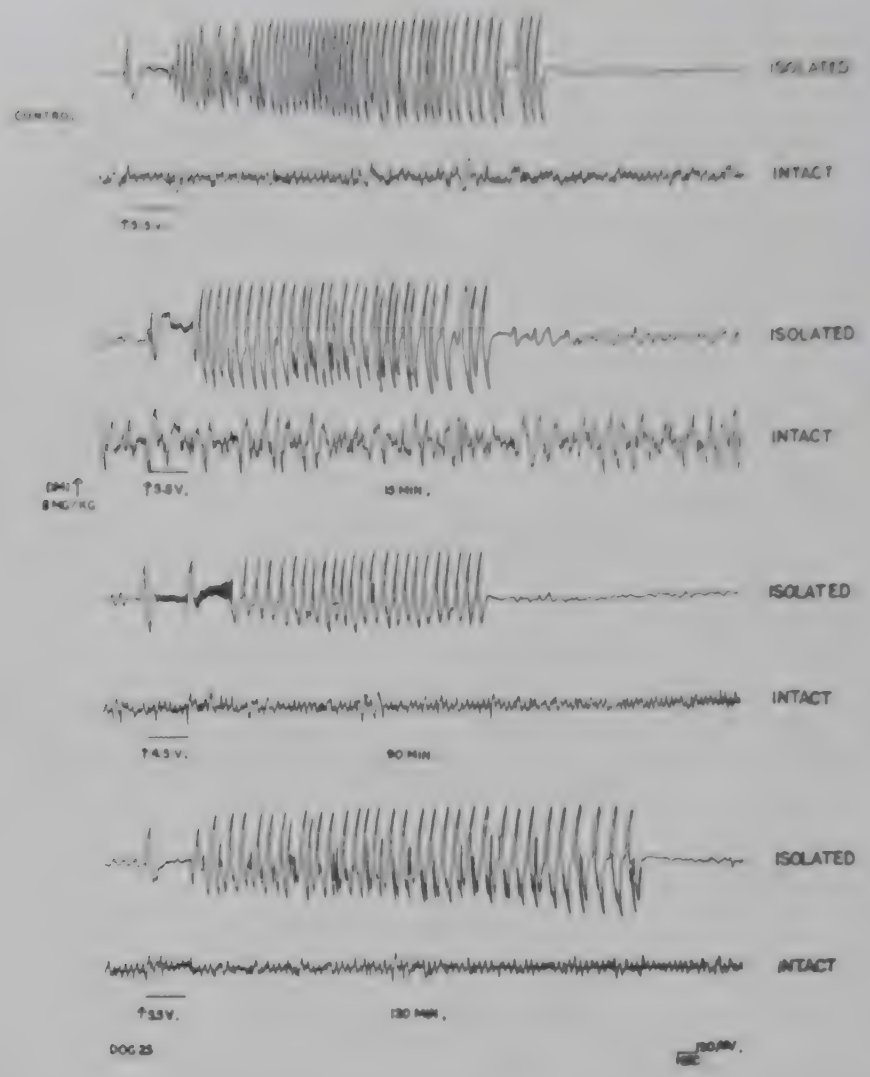

FIG. 1 — EFFECT OF DESMETHYLIMIPRAMINE (8·0 mg./kg. i.v.) ON THE SPONTANEOUS ACTIVITIES OF INTACT AND ISOLATED CORTICES AND ON THE THRESHOLD OF THE AFTER-DISCHARGE IN ISOLATED CEREBRAL CORTEX [At 15 min. it induced high voltage slow wave activity in both the cortices, while the threshold of the after-discharge remained unaffected. However, 90 min. after the drug administration, it induced low voltage fast wave activity in the corticograms and lower the threshold of the after-discharge from 5·5 V. in the control to 4·5 V. Recovery is seen at 120 min.]

recovered in 120 min. yet the duration of the after-discharge remained increased.

Fig. 2 shows the effect of DMI (8·0 mg./kg. i.v.) on the duration of the after-discharge at a fixed submaximal voltage. At 15 min. after the drug administration, the duration of the after-discharge was not affected but at 90 min. it was markedly increased. Recovery from this effect could not be observed up to 120 min.

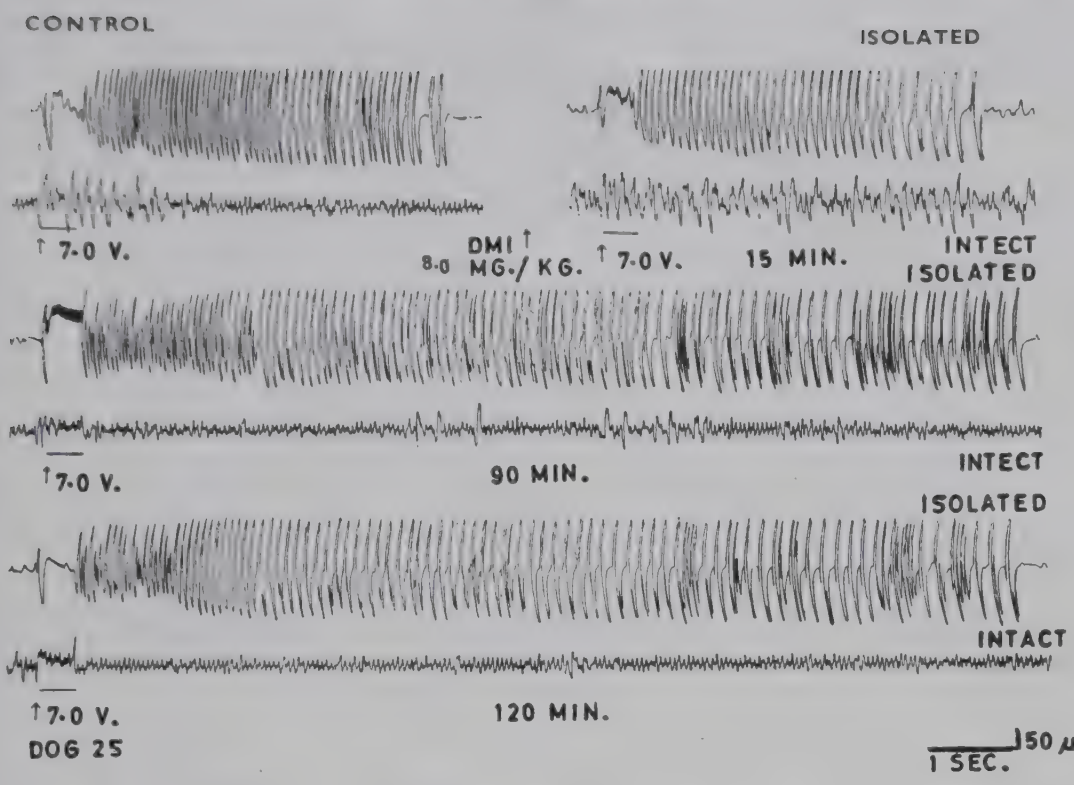

FIG. 2—EFFECT OF DESMETHYLIMIPRAMINE (8·0 mg./kg. i.v.) ON THE DURATION OF EVOKED AFTER-DISCHARGES AT FIXED SUBMAXIMAL VOLTAGE (7·0) [Note 15 min. after the drug, there is no change in the duration of the after-discharge. After 90 min. the duration is markedly increased. Recovery could not be seen up to 120 min.)

Somatic Reflex Studies

Sensitivity of patellar monosynaptic reflex to imipramine. Effect of intrathecal imipramine (1·0–2·0 mg.) has been studied on the patellar monosynaptic responses in spinal cats. Imipramine depressed the amplitude of the patellar reflex. The inhibitory effect appeared within 5–7 min., reached its peak in 15 min. and lasted for 70–90 min. depending upon the dose. Doses less than 1·0 mg. did not depress the reflex.

Sensitivity of the polysynaptic reticular facilitation of patellar reflex to imipramine. Polysynaptic reticular facilitation of the patellar reflex was elicited in seven cats under chlorolose anaesthesia. Stimulation (3–5 V., 120 shocks/sec. for 15 sec.) of the facilitatory area of the reticular formation elicited an increase in the amplitude of the patellar responses as well as vasopressor responses. Local application of imipramine (1·25%) at the site of the electrode, reduced the amplitude of the patellar reflex, abolished the reticular facilitation and depressed the vasopressor responses. Only partial recovery from the drug effect was observed at 90 min. Fig. 3 shows the record of one such experiment.

Sensitivity of the polysynaptic reticular inhibition of patellar reflex to imipramine. Polysynaptic reticular inhibition of the patellar

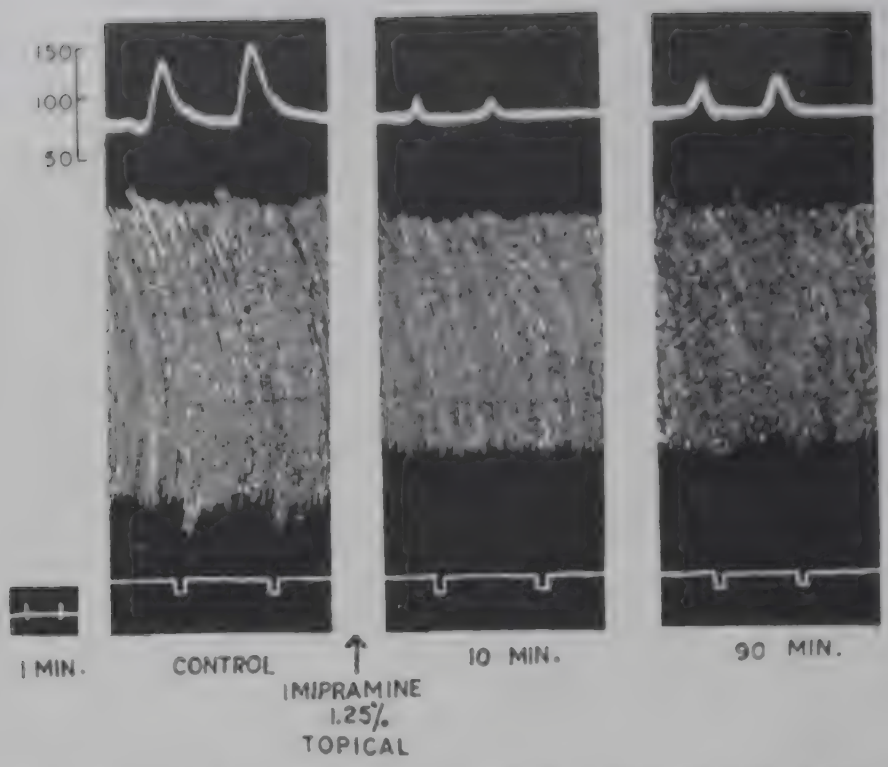

FIG. 3 — EFFECT OF IMIPRAMINE ON BLOOD PRESSURE (UPPER TRACING), PATELLAR TAP RESPONSES EVERY FIVE SEC. (LOWER TRACING) AND POLYSYNAPTIC FACILITATION ON THE PATELLAR REFLEX DUE TO THE RETICULAR FORMATION STIMULATION (3·0 V., 100 shock/sec. for 15 sec.) [Note topical application of imipramine (1·25%) reduced the amplitude of the patellar reflex and abolished the reticular facilitation of the patellar reflex 10 min. after the drug administration. Recovery occurred in 90 min.]

reflex due to stimulation (0·25–0·5 V., 120 shocks/sec. for 15 sec.) of reticular inhibitory area was obtained in four cats. Fig. 4 shows the effect of topical imipramine (1·25%) on the PR and its inhibition. It reduced the amplitude of the patellar reflex and augmented the reticular inhibition. Recovery was observed in 80 min.

Relative sensitivity of linguomandibular polysynaptic reflex (LMR) and patellar monosynaptic reflex (PR) to imipramine. Effect of intravenous imipramine (2·0 mg./kg. i.v.) was studied simultaneously on the linguomandibular polysynaptic reflex (LMR) and the patellar monosynaptic reflex (PR) in the same preparation. Imipramine selectivity depressed the linguomandibular reflex mediated at the brain-stem level (Fig. 5).

DISCUSSION

Despite extensive neuropharmacological researches the precise mechanism of the antidepressant action of imipramine is not clear. Sulser *et al.* (1962, 1964) have suggested that the characteristic antidepressant action

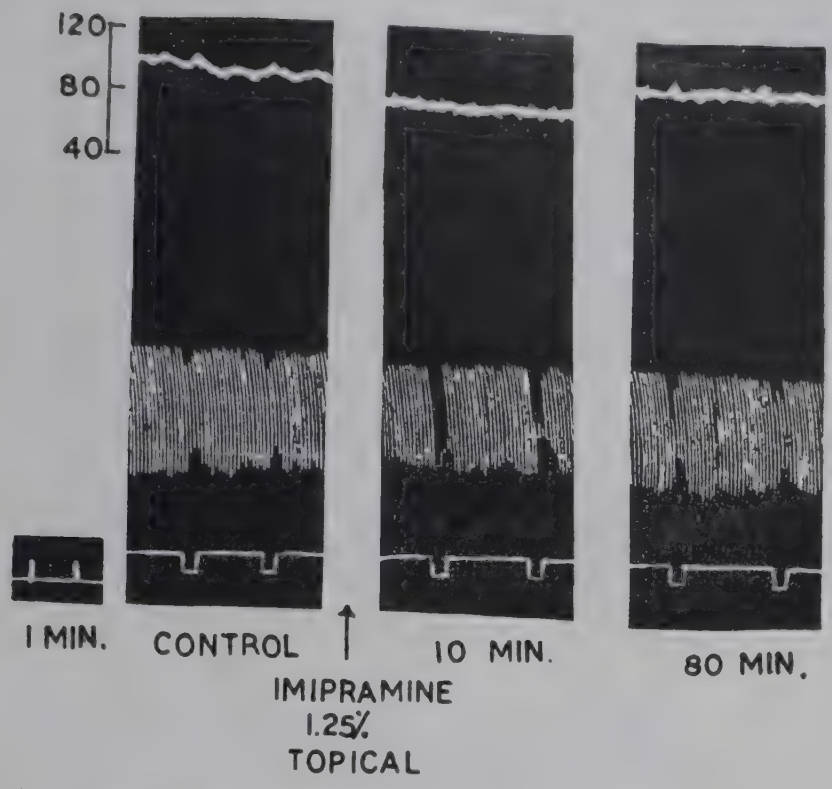

FIG. 4—EFFECT OF IMIPRAMINE (1·25%) ON BLOOD PRESSURE (UPPER TRACING), PATELLAR TAP RESPONSES (LOWER TRACING) AND POLYSYNAPTIC INHIBITION OF PATELLAR REFLEX DUE TO RETICULAR FORMATION STIMULATION (0·5 V., 100 shocks/sec. for 15 sec.). [Note reduction in amplitude of the patellar reflex and enhancement of inhibition of patellar reflex to reticular stimulation, 10 min. after topical application of the drug. Recovery from drug effect obvious at 80 min.]

of imipramine is mediated through its demethylated metabolite desmethyl imipramine (DMI). Their suggestion is based only on the antagonism of reserpine syndrome exhibited by imipramine. A satisfactory explanation has not yet been put forth for the antidepressant action of imipramine.

In the present investigation an attempt was made to study the effect of imipramine on the electrocortical arousal due to mid-brain reticular stimulation, the spontaneous and evoked activities of the isolated cerebral cortex and the facilitatory and inhibitory influences of the brain-stem reticular formation on the somatic reflexes. From the results it is clear that imipramine exerted a depressant effect both at the reticular as well as the cortical level up to six hours. However, a delayed 'stimulant' effect with higher doses of imipramine was observed after 375 min. on the neuronal excitability of the isolated cerebral cortex preparation. The delayed stimulant action could be due to metabolic conversion of imipramine into desmethyl imipramine. DMI was found to exert a potent stimulant effect both at the reticular as well as the cortical level. This stimulant effect on the cortical neurones appeared after 90 min. and lasted up to 200–50 min. depending upon the dose.

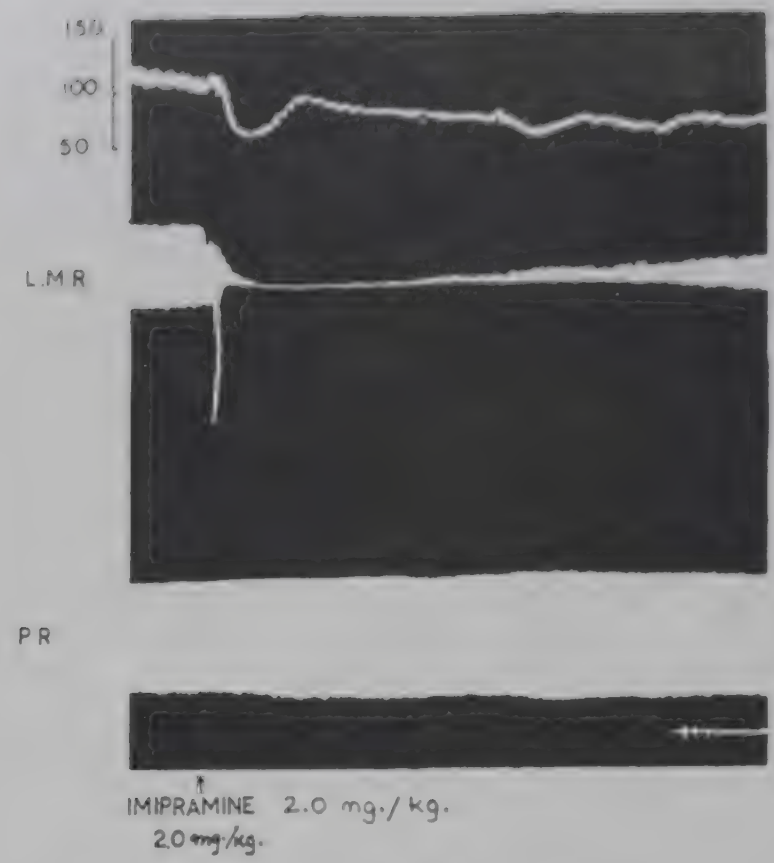

FIG. 5—EFFECT OF IMIPRAMINE (2·0 mg./kg. .v.) SIMULTANEOUSLY ON BLOOD PRESSURE (UPPER TRACING), POLYSYNAPTIC LINGUOMANDIBULAR REFLEX (LMR, MIDDLE TRACING) AND MONOSYNAPTIC PATELLAR REFLEX (PR, LOWER TRACING) [Note imipramine depressed the linguomandibular reflex without affecting the patellar reflex]

The fact, that the stimulant effect appears earlier and is of greater potency with DMI, suggests that DMI might be the active metabolite for the antidepressant action of imipramine. This observation also explains the delay observed in the appearance of its antidepressant effect in patients suffering from endogenous depression.

Imipramine group of drugs have been found to antagonize the increased muscle tone of reserpine syndrome [Sulser *et al.*, 1962] and inhibit the linguomandibular polysynaptic reflex (Sinha *et al.*, 1966a).

Orphenadrine, another antidepressant having structural similarity to imipramine, has been shown to possess central muscle relaxant property [Biplasma *et al.*, 1956]. In the present study imipramine has been found to exert an inhibitory effect on the supraspinal and the spinal influences on the somatic reflexes. The inhibitory effect of imipramine on the muscle tone might be playing some role in the antidepressive action of imipramine.

From the present study it is clear that imipramine exerts a depressant action at all the levels of the neuraxis, similar to chlorpromazine to which

it is chemically related. Thus imipramine is primarily a tranquillizer like chlorpromazine. It is only after the metabolic transformation of imipramine into DMI that its antidepressant action is unmasked.

ACKNOWLEDGEMENT

The authors are grateful to Messrs J. R. Geigy, Switzerland for the supply of imipramine (Tofranil) and desmethylimipramine (Pertofran) and to the Council of Scientific & Industrial Research for providing financial assistance.

REFERENCES

1. Bhargava, K. P. & Srivastava, R. K., *Br. J. Pharmac.*, **23** (1964), 391.
2. Bhargava, K. P. & Srivastava, R. K., *Br. J. Pharmac.*, **25** (1965a), 751.
3. Bhargava, K. P. & Srivastava, R. K., *Br. J. Pharmac.*, **25** (1965b), 74.
4. Bijlasma, U. G., Harms, A. F. Funcke, A. B. H., Tersteege, H. N. & Nauta, W. T., *Arch. int. Pharmacodyn.*, **106** (1956), 332.
5. Calma, I. & Wright, S., *J. Physiol.* (Lond.), **106** (1947), 211.
6. King, E. E. & Unna, K. R., *J. Pharmac. exp. Ther.*, **111** (1954), 293.
7. Kristiansen, K. & Courtois, G., *Electroenceph. Clin. Neurophysiol.*, **1** (1949), 265.
8. Kuhn, R., *Schweiz. med. Wschr.*, **87** (1957), 1135.
9. Kuhn, R., *Am. J. Psychiat.*, **115** (1958), 459.
10. Osborne, M. & Sigg, E. B., *Arch. int. Pharmacodyn.*, **129** (1960), 273.
11. Saxena, P. N., Tangri, K. K. & Bhargava, K. P., *Electroenceph. Clin. Neurophysiol.*, **17** (1964), 506.
12. Sinha, J. N., Srimal, R. C., Dixit, K. S., Om Chandra & Bhargava, K. P., *Jap. J. Pharmac.*, **16** (1966a), 120.
13. Sinha, J. N., Tangri, K. K. & Bhargava, K. P., *Electroenceph. Clin. Neurophysiol.*, **21** (1966), 301.
14. Sulser, F., Bickel, M. H. & Brodie, B. B., *J. Pharmac.*, **144** (1964), 321.
15. Sulser, F., Watts, J. & Brodie, B. B., *Ann. N.Y. Acad. Sci.*, **96** (1962), 279.

Morphine-like Acting Compounds, Protective System and Drug Addiction (Dependence)[1]

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Considering that analgesic action of morphine is by far the most important of its effects, it is surprising that in the great review on pharmacology of morphine by Krueger and coworkers[2] in 1941, only one per cent was concerned with analgesia and more than 99 per cent of the work was devoted to the so-called side effects.

There is a simple explanation for this strange disproportion. Until my discovery[3] of the morphine-like action of simple synthetic compounds, at first pethidine, morphine itself and some of its derivatives were our only powerful weapons in the struggle against pain.

With the large number of synthetic analgesics now available it became possible to separate the specific from the nonspecific effects[4]. An effect is specific if it is common to all analgesics, independent of their chemical structure, and if the potency in producing the effect runs parallel to their analgesic potency.

Another criterion for the specificity of an action is its great sensitivity against small changes in the chemical constitution. For instance the analgesic effect of methadone is abolished by an exchange of the methyl group in the side chain for an ethyl group and that of pethidine by substituting a hydroxy group in *ortho* or *para* position but not in *meta* position.

A particularly striking example in that respect is also the great difference of the activity between optical isomers. In methadone the dextro-isomer is at least 30 times less potent than the levo-isomer.

Finally, all specific effects of morphine-like acting compounds (MLC) are antagonized by nalorphine.

Paper presented in absentia

Based on these criteria, there are the following effects of MLC specific ones: (i) analgesia, (ii) the depression of respiration, (iii) the effect on intestinal motility, (iv) a central excitation in cats and mice, (v) the hyperglycaemic effect, (vi) the effect on body temperature and finally, the liability to produce addiction, and psychical and physical dependence.

All these effects are inseparable from the analgesic action and it is not correct to say that they are side effects. This is unfortunately also true for the liability to produce addiction and dependence. It will therefore not be possible to find MLC without this undesirable addiction. In fact all efforts in this direction have been and will be unsuccessful.

Examples for unspecific actions of the MLC are the toxicity and the spasmolytic action on smooth muscles.

Pethidine, for instance, is twice as toxic as morphine in the mouse but about seven times less active in analgesic action. Otherwise, pethidine is an excellent spasmolytic acting compound but morphine is without any activity in this respect.

The parallelism between the specific activities sugg sts that there exists also a close relationship between the different physiological functions which are affected by MLC. In my opinion, this really is the case. All these functions have the common task of warning and protecting the organism against imminent damage; they exert a protective function.

There can be no doubt that pain in all its variations has such a protective function.

In analogy the warning sensation of dyspnoea which can reach the terrible feeling of suffocation protects the body against acidosis.

There is a similar safeguard concerning circulation. The disagreeable throbbing of the heart and the feeling of anxiety which can develop into agony associated with stenocardia warns against imminent collapse by overstrain of the heart.

The osmotic pressure of the body fluids must be exactly constant. A little increase over the normal range produces the torment of thirst.

Other warning and protecting sensations are the feeling of cold or of unbearable heat if the body temperature can no longer be kept within the normal range by the autonomic regulations.

These physiological functions can be separated from the rest of autonomic regulations and from the epicritical system of sensual perception because of their special task and because of their specific sensitivity to the MLC. They can be considered together as forming what I have termed the protective system[5].

It is characteristic of this protective system that its different functions are brought into action by noxious stimuli and that in man they enter the psychic sphere. All these separate functions are inhibited by the MLC in proportion to their analgesic potencies. Analgesia is therefore only one part of a general antiprotective action of the morphine-like analgesics.

Doses of the MLC which suppress the dysphoric sensations connected with the functions of the protective system do not influence sensory perceptions of the epicritical system like touch, taste, vision and hearing and their mental assimilation. In this respect MLC contrast with general anaesthetics. The invaluable relief given by the MLC rests on their ability to suppress the protective system with its dysphoric sensations and anxiety, on their specific antiprotective action.

But the higher psychic sphere of the human being is guided and ruled by dysphoric warning sensations too; there exists also, to a certain extent, a psychic protective system.

Bad conscience, inferiority complex, anxiety concerning the struggle of life are symptoms of an excitation of the psychic protective system and can produce a general tormenting mental agony. These dysphoric sensations of quite pscychic origin are decreased and abolished by MLC too.

The physical and psychic antiprotective effect together leads in this way, by abolishing all dysphoric sensations, to a true euphoria.

But the delivering action of the MLC implies also the danger of reducing to abuse which ends in addiction and dependence.

Decisive for that irresistible fate of the chronic abuse are the increasing tolerance which leads to increase the doses and the symptoms of withdrawal which lead to the compulsive continuation of abuse.

Both increasing tolerance and withdrawal symptoms have the same reason. A chronic damping of the protective system causes a compensatory increase of its function which in itself forces an increased dose. After a longer period of that vicious circle, the sudden interruption of the medication the compensatory hyperfunction of the protective system which is now no longer damped by the antiprotective effect of the MLC produces the symptoms of withdrawal with its terrible agony. A repeated dose of MLC will abolish the withdrawal symptoms at once.

At that moment the stage of dependence is attained. The addicts now yield to an inherent psychic drive not under their control[6]. Therefore they may not be classified as criminals; they are pitiably ill persons.

If such a dependence is provoked by therapeutical prolonged application of increasing doses of MLC in patients with a normal protective system this will be restored to a normal sensitivity after withdrawing the drug and subsiding of the withdrawal symptoms. The dependence will be abolished for ever. However, if the use of MLC was induced in persons with a primary neuropathic hypersensitivity of the protective system the dysphoric sensations provoked by the normal struggle of life will reappear. These patients cannot be cured for good from their dependence simply by withdrawing the drugs without rehabilitating psychotherapy. Otherwise they will or must relapse.

For the therapeutic use, the MLC are more and at the same time also less than analgesics. Less, because in clinical doses they do not abolish the sensation of pain completely, as was demonstrated experimentally in

human beings. They are more than analgesics, because they do abolish the mental processing of pain which leads to the tormenting feeling or experience of pain. Therefore it would be better to term the MLC as analgothymics or analgognosics than as analgesics.

Administering MLC during a short period in patients not suffering from a congenital or acquired hyperfunction of the protective system will never lead to dependence. In order to prevent the development of tolerance and dependence during prolonged administration of MLC, the dosage must be kept as low as possible. One should not be tempted by an unsatisfactory analgesic effect to increase the dose unsuitably. Even doubling the dose may not improve the result. It is the so-called ceiling effect. To get a true and complete analgesia it would be necessary to give the ten-fold clinical doses which is only possible in experiments with animals.

The best way to relieve severe pain in spite of a low dosage of MLC would be to combine the suppression of processing of pain, the algothymia or algognosia, by MLC with a suppression of pain sensation, the algesia, by a nonaddicting analgesic, for instance amidopyrine, acetylsalicylic acid, acetophenetidine, etc. to complete 'analgothymia' by 'analgesia'. It may be left to the skill of the doctor to select the best analgesic corresponding to the cause of pain. Such combination will be the best way towards an optimal control of pain with the lowest possible danger to produce addiction also over a prolonged period of time.

In that respect methadone may be one of the least dangerous MLC[7]. In therapeutical use, tolerance develops only slowly. Withdrawal after prolonged administration will meet no great difficulties since withdrawal symptoms are relatively mild and slow in onset in comparison with morphine. Therefore, in curing addicts also the best method is at first to substitute morphine or heroin by methadone and then withdrawing this.

A really very great danger to the society is the addiction to MLC, which is widespread only in the USA. The reason for it is the very well organized class of black marketeers. The main danger there is not only their supplying the required drug to addicts at exhorbitantly high prices but even more, they attempt to increase their turnover, which is about 250 million dollars a year, by enlarging the number of customers.

In Europe, there is no black market at all. Therefore, the number of addicts is very small; there, alcoholism has assumed dangerous proportions.

In both these respects, in my opinion, India is a happy country. Neither MLC, above all morphine and heroin, nor alcohol is in abuse. Important for India, I think, is the smoking and eating of opium. There may result, by a widespread abuse, to some extent an addiction but seldom a true dependence, because in this way of consumption the daily consumed doses cannot exceed a certain limit. The outstanding damage to the community by smoking opium is the severe impairment of social productivity.

REFERENCES

1. [illegible], O.: Morphin- u. morphinartig wirkende Verbindungen. Handbuch d. exp. Pharmakologie, Ergänzungsband 12; (1957).
2. [illegible]
3. Schaumann, O., *Arch. exp. Path. u. Pharmakol.*, **196** (1940), 109.
4. Schaumann, O., *Arzneimittelforschung.*, **4** (1954), 115.
5. Schaumann, O., *Naturwissensch.*, **41** (1959), 96; Dtsch. med. Wschr. 1954, 157.
6. Seevers, M. H., *N.Y. Acad. Sci.*, **51** (1948), 98.
7. Seevers, M. H., *Bull. on Narcotics*, **8** (No. 2), (1956), 26.

CNS Drugs in Experimental Cancer Research

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Over the years, the signs have accumulated, as much in the domain of the pathophysiology of cancer as in that of clinical experience, pointing to the fact that in many, if not indeed in the majority, of spontaneous cancers the proliferation of malignant cells (i.e. the process through which the presence of these abnormal cells in the body becomes a disease) is under the constant influence of multiple environmental factors within the organism. One of these factors which recently has passed from the realm of speculation to the phase of active experimental research, is the role played by the central nervous system in the evolution, and possibly in the induction of certain tumours. This, I think, must certainly have been in the mind of the organizers when they so kindly invited me to participate in this important symposium devoted to the pharmacology of CNS drugs. For, where and when the growth of a tumour can be proven to be to some extent under the control of the nervous system, there is there a rationale for the cancerologist to investigate the effects of CNS drugs in his own field.

The most significant advances in our knowledge of cancer dependence on the nervous system have arisen from a new understanding of the physiology of the sex organs. The pituitary gland, which was once considered as the highest point of control of the morphogenesis, development, and functioning of the primary and secondary sex organs through the secretion of hypophysial stimulins, has now been shown to be itself under the dominion of the hypothalamic centres. As is known, the hypothalamus exerts its influence on the anterior pituitary by means of releasing-factors of a chemical nature which regulate the output of the pituitary stimulins[1]. These in turn control among other biologically active substances the production of steroid hormones by the gonads and the adrenal cortex and recently, Wurtman and

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Axelrod[1] produced evidence that even the synthesis of adrenalin in the adrenal medulla comes under the control, at least in large part, of the pituitary gland via the adrenocortical hormones, which activate phenylethanolamine-*N*-methyltransferase, the enzyme responsible for the methylation of noradrenalin to adrenalin.

In a reverse manner, the activity of the hypothalamus can be modified, through feed-back mechanisms, by the hormones produced by the target-glands, e.g. ovaries, testicles, etc. (this is the so-called long-route feed-back), or, more directly, by the pituitary itself (the so-called short-route feed-back). In addition to these hormonal feed-back, there is of course the purely neural feed-back which is triggered off by the external stimuli picked up by the genital tract and mammary glands.

Besides the hypothalamus, it has recently been established that the pineal gland can bear a strong influence on the gonads, especially in female animals, and that this influence is mediated by melatonin, an indolic hormone whose biosynthesis seems to be peculiar to this gland and is in turn regulated by the stimuli produced by light, which act on the pineal body through sympathetic nerve fibres via the superior cervical ganglia. In their brilliant

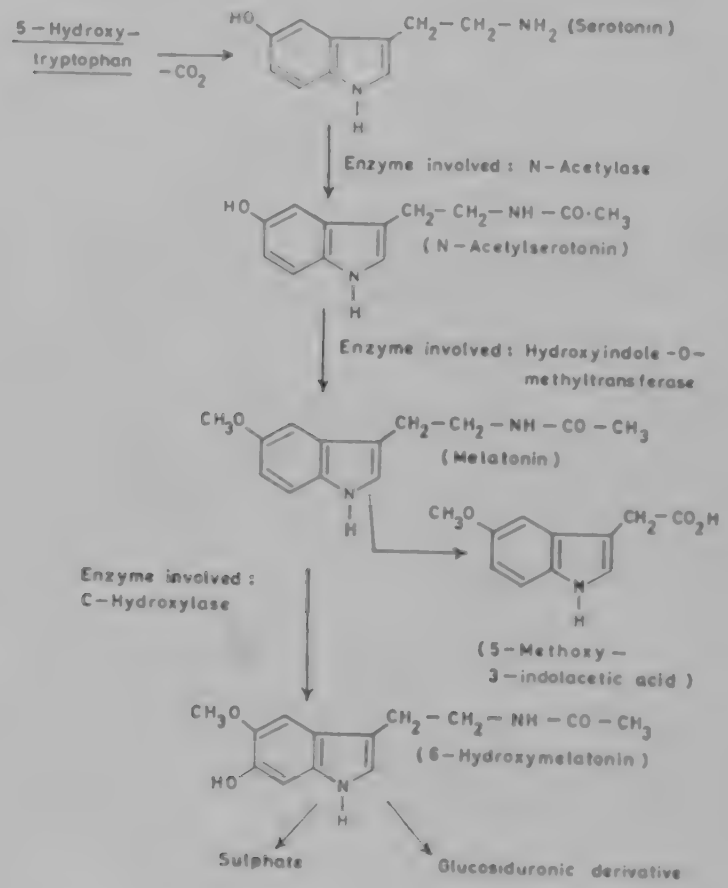


FIG. 1 — BIOSYNTHESIS AND DESTRUCTION OF MELATONIN (Axelrod *et. al.*, 1965)

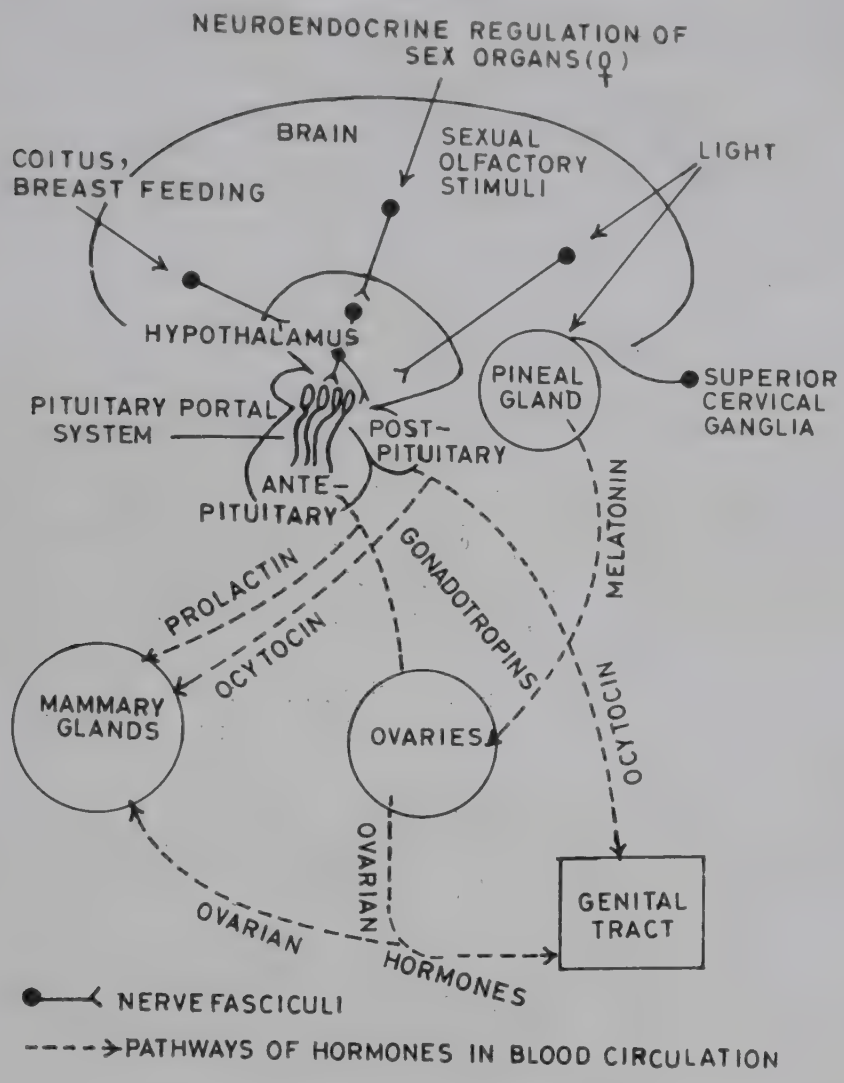


FIG. 2 — NEUROENDOCRINE REGULATION OF SEX ORGANS

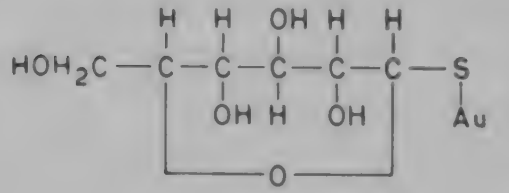
work, Axelrod and his collaborators[3] have demonstrated that melatonin inhibits oestrus, decreases ovary weight, and is highly concentrated from the blood stream by the pineal gland, the iris-choroid, and the ovary. These findings suggest the existence of a feed-back mechanism for melatonin too. Figure 1 shows the various steps observed in the biogenesis of this new hormone from serotonin, and its degradation to inactive products.

Figure 2 gives a diagrammatic representation of those features of neuroendocrine regulation of the sex organs which have been experimentally well established and which are the most important for cancer research.

To this firm pattern of neuroendocrine regulation of the sex organs, there must probably be added the participation of less well-known areas of the brain such as the rhinencephalon and the *substantia reticulata*, both of which seem also to be particularly sensitive to small osmotic, ionic and hormonal changes occurring in the internal milieu, and transmit the

information to the hypothalamus itself, thereby influencing, in an indirect manner, the anterior lobe of the pituitary.

In Fig. 2, the organs in females which are particularly susceptible to cancerization (mammary glands, uterus, ovaries) are shaded in and it is the tumours which appear in these organs that reveal themselves to be the most responsive to neuroendocrine influences, both in experimental carcinogenesis and in human cancer chemotherapy. It is not surprising, therefore, that it is on such tumours that the effect of certain drugs with action on the central nervous system has been most easily demonstrated. In this respect, one of the most interesting categories of substances is that of compounds which, on injection, are capable of producing anatomic lesions in the areas of the nervous system which are important for neuroendocrine regulations. The most investigated compound of this series is gold thioglucose (I), a drug which had been used as an antituberculous agent under the name of Solganal B, and which Marshall and his collaborators showed could produce considerable destruction to the hypothalamic area by intraperitoneal injection in certain strains of mice[4]; these lesions result in the rapid appearance of mammary carcinomas, even in male animals[5]. A more recently discovered drug, polybrene, or hexadimethrine bromide, a polymer having formula (II), and which is an antagonist of heparin, is able to produce necrotic lesions on a lower level, that is to say in the pituitary (and in the adrenals), and its usefulness in cancer research is currently being investigated by Perrault in Paris. A more common group of substances which can affect mammary cancer is that of the neuroleptics. Particularly interesting among these is the alkaloid reserpine (III), whose well-known action on the subcortical zone, leading to the inhibition of the hypothalamic centre of control of



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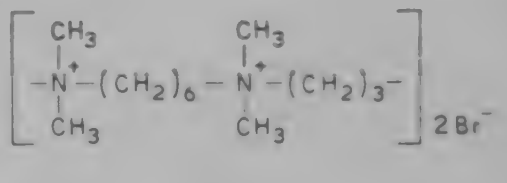

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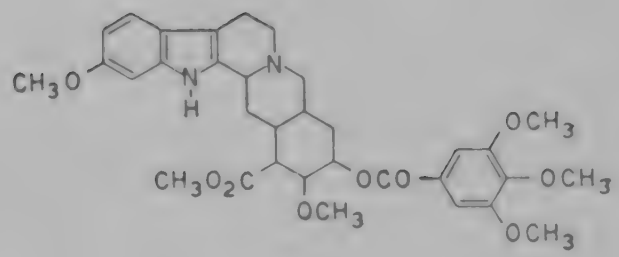

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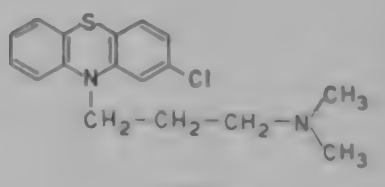


(IV)

prolactin secretion, induces in female rats a marked lobulo-alveolar development of the mamma and the secretion of milk[6]. Lacassagne and his coworkers[7] showed that this effect of reserpine on the hypothalamus causes in mice the production of persistent false corpora lutea and in a strain of mice in which spontaneous mammary cancer occurs, an increase in both the incidence and the speed of induction of these tumours.

Another organ which is a target for the steroid hormones coming from the gonads and the adrenals is the liver, which at the same time is the main centre for the metabolization of steriod compounds in the organism. The dependence of primary cancer of the liver on neuroendocrine factors is particularly easy to investigate, as experimental hepatocarcinogenesis in rats by means of chemical substances such as *p*-dimethylaminoazobenzene (DAB) and similar azo compounds has become a routine laboratory procedure, and the various phases of histopathological changes in the process of cancerization have been studied and described in every detail by Lacassagne and his coworkers[8]. In the case of DAB-carcinogenesis, which is the best known, these phases follow a sequence starting from a precocious degeneration of scattered hepatocytes, to the ultimate appearance of multicentric carcinomas passing through a stage of bile duct proliferation. DAB-cancerization is prevented by hypophysectomy[9], this being due to the interruption, in the chain of regulation of liver regeneration, of the secretion of ACTH, as is shown by the fact that adrenalectomy also hinders this type of carcinogenesis[10]. Fig. 3 represents a tentative picture of the various factors which make up the neuroendocrine regulation of the liver. The corticotropin-pituitary secretion being controlled by the hypothalamus, cancer of the liver by DAB lends itself especially well to the study of possible effects of CNS drugs on one type of cancerization. This work has been pursued systematically at our Institute, under the direction of Lacassagne for a number of years. In 1959, Lacassagne, Hurst and Rosenberg[11] demonstrated that reserpine, even in the relatively low dose of 80 mcg. per animal and per day, produced in rats a striking acceleration of DAB-carcinogenesis, and cancers were obtained as early as after 70 days, whereas in the controls, and with the semi-synthetic diet used, the first tumours appeared only after 5 months. In similar experiments, chlorpromazine (IV) has also shown a synergistic effect with DAB, but to a considerably milder degree than reserpine. In these experiments, reserpine and chlorpromazine exerted their effects through an inhibition of the hypothalamic centres of prolactin- and ACTH-regulation.

Other drugs with activity on the central nervous system have also been investigated in relation to DAB-carcinogenesis, and interestingly enough, some of them have been found to exert on this malignant process an effect which is diametrically opposed to that of reserpine and chlorpromazine. One compound often investigated is *p*-hydroxypropiophenone (PHP) (V). The mechanism of action of this drug which is widely used clinically as a

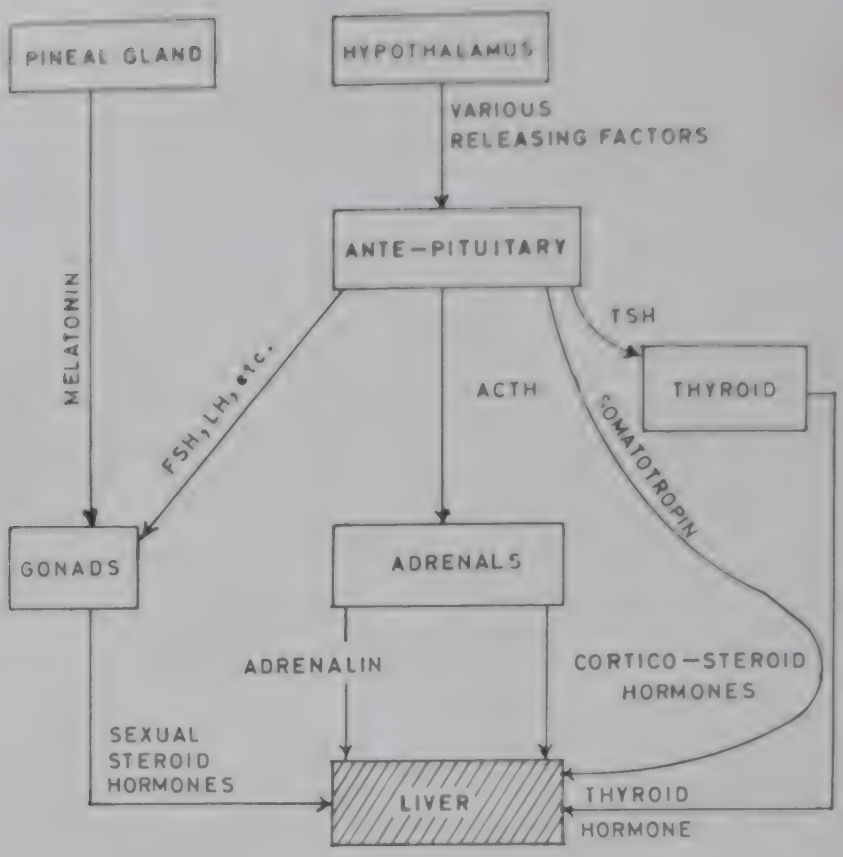
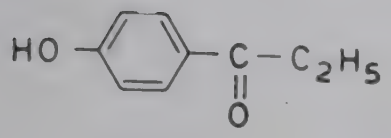


FIG. 3 — NEUROENDOCRINE REGULATION OF THE LIVER (tentative picture)

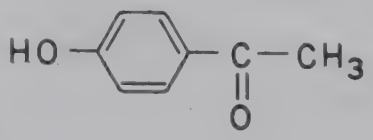
moderator of the pituitary function, for a long time a matter of controversy, has been clarified in recent experiments.

In particular, Iwasawa, using tadpoles of Japanese frog *Rana temporaria ornativentris*, showed that administration of PHP suppressed the considerable acceleration in gonadal development induced by thyroidectomy, this action of PHP being accompanied by the phenomenon of sex reversal from male to female which occurs in hypopituitarism[12]. In higher animals, the depression of gonadotropin secretion which PHP brings about can be traced to a reduction in the ovarian activity, by following the sex cycle in females[13]. That the moderating effect of PHP on the antipituitary might be one that is exerted on the hypothalamic centres has been suggested by experiments performed recently in France on the activity of the superior nervous zones of the brain in the female rabbit[14].

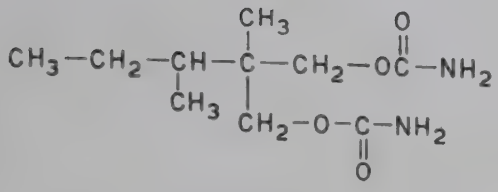
In liver carcinogenesis, using PHP, Robertson and his coworkers[15] and later the Japanese cancerologist Baba[16], were able to inhibit cancerization by DAB. Lacassagne and his coworkers[17] found a similar inhibitory effect in a series of homologues and derivatives of *p*-hydroxypropiophenone, among these, a particularly potent compound was *p*-hydroxyacetophenone (VII), which suppressed completely the oncogenic activity of DAB. All these



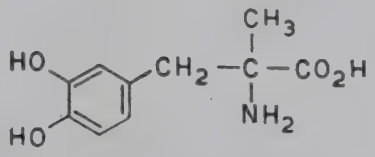
(V)



(VI)



(VII)



(VIII)

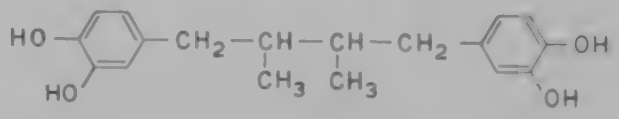
compounds bear a common skeleton, which is that of acetophenone, whose action on the central nervous system is well known.

Quite recently, we have extended this type of research to further families of CNS drugs, including carbamate esters of the meprobamate group such as 'Capla' (VII), and inhibitors of aromatic amino acid-decarboxylase such as α-methyldopa (VIII). These experiments are not yet complete, but as regards the early phases of DAB-intoxication, a protective effect has already been noted.

Apart from the areas of the central nervous system with which I have dealt in detail, their functions having to a greater or lesser degree been clarified, there is little doubt that other sectors of the CNS, and indeed also of the autonomous nervous system, must contribute their share to the maintenance of the mitotic and functional homeostasis of the body. Since the time of Charles Sherrington, much information has been acquired on the ways the nervous system performs what the great physiologist termed its 'integrative action'[18]. For instance, we know now that the activity of several enzymatic systems is controlled by sympathetic nerves, this is the case of 5-hydroxytryptophan decarboxylase, which, in the pineal gland, manufactures the serotonin needed for the subsequent biosynthesis of melatonin[19]. Other chemical effectors of the nervous system such as acetylcholine are known to induce hypothalamic-hypophysial neurosecretion, by remote injection in experimental animals[20]; the role of catecholamines as 'neuro-humours' must also be emphasized.

But all the biochemical transformations activated by the nervous system are not necessarily beneficial to the maintenance of homeostasis. A striking example of this is provided by the recent observation of Horgan and Philpot[21] that in mice submitted to nervous strain through fright, abnormal amounts of peroxide-like substances are produced in the body; to this response to nervous stress which consists in the endogenous production of cytotoxic

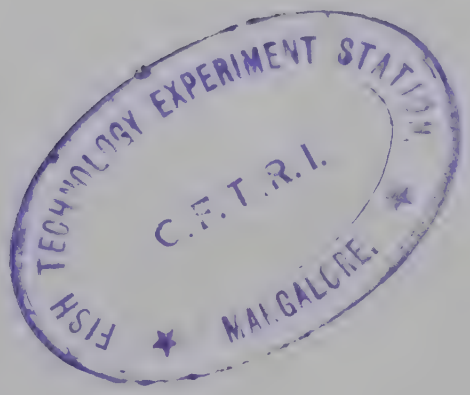
compounds, I might venture to correlate the well-known phenomenon often reported by clinicians, of an acceleration of tumour growth in the wake of nervous disaster. This biochemical interpretation of certain pathological consequences of nervous shock, if it were to be proven beyond doubt, would open up a wholly new approach to the chemotherapy and chemoprophylaxis of the organic disorders produced by stress, based on the use of powerful antioxidants. In this regard, α-tocopherol could then find a wider use than merely as vitamin E, especially as it has been claimed by the Russian physiologist Uskov[22] to have, in addition, pronounced ataractic properties in doses of 0.5 to 1 g./kg. In my own experiments, *d, l*-α-tocopherol acetate did show a potentiating effect on hexobarbital-induced hypnosis in mice (although the very high dose of 10 g./kg. was necessary), this effect being expressed in the lengthening of the sleeping time: for an intravenous dose of 40 mg./kg. of sodium hexobarbital, which induced hypnosis in 100 per cent of the animals, the average sleeping time was 8 min. in the controls and 13 min. in the animals given the α-tocopherol acetate subcutaneously 3 hr before barbiturate administration; with 60 mg./kg. of sodium hexobarbital, the average sleeping time, which was 24 min. in the controls, was extended to 30 min. in the treated animals. Doses of 1 g./kg. of tocopherol, however, had no effect. Ataractic effects have also been found in another natural antioxidant, nordihydroguaiaretic acid (IX).



(IX)

It is perhaps pertinent to mention here the many experiments pointing to a significant decrease in the incidence of methylcholanthrene-induced tumours in mice through simultaneous administration of chemical reductors, which included sodium nitrite, sodium cobaltinitrite, and α-tocopherol itself[23]. These substances have in common the property of working against peroxides and experiments are under way in our laboratory to see to what extent, and how, the chemical induction of cancer can be affected by high tissular levels of organic antioxidants.

REFERENCES

1. Lacassagne, A., *La Presse Medicale*, **69** (1961), 2285.
2. Wurtman, R. J. & Axelrod, J., *Science*, **150** (1965), 144.
3. Wurtman, R. J. & Axelrod, J., in Progress in Brain Research, Vol. 10, (1965), p. 327, Elsevier Pub. Co., Amsterdam, 520.
4. Marshall, N. B., Barnett, R. J. & Mayer, J., *Proc. Soc. exp. Biol. Med.*, **90** (1955), 240.
5. Liebelt, R. A., *Proc. Am. Ass. Cancer Res.*, **3** (1959), 37.
6. Benson, G. K., *Proc. Soc. exp. Biol. Med.*, **99** (1958), 550; Keil, R., Aeschbert, R., Gage, C. & Amarger, J., *C. R. Soc. Biol.*, **150** (1956), 981.

7. Lacassagne, A., Duplan, J. F. & Buu-Hoi, N. P., *C. R. Acad. Sci.*, **247** (1958), 629; Lacassagne, A. & Duplan, J. F., *ibid.*, **249** (1959), 810.
8. Lacassagne, A., Berliner Symposion über Fragen der Carcinogenese, Akademie-Verlag, Berlin, 1960, p. 169.
9. Robertson, C. H., O'Neal, M.A., Griffin, A. C. & Richardson, H. L., *Cancer Res.*, **13** (1953), 776.
10. Symeonidis, A., Mulay, A. & Burgoyne, F. H., *J. natn. Cancer Inst.*, **14** (1954), 805.
11. Lacassagne, A., Hurst, L. & Rosenberg, A. J., *C. R. Acad. Sci.*, **249** (1959), 903.
12. Iwasawa, H., *Endocrinologia Japonica*, **7** (1960), 181.
13. Buriana, L. M. Ciurea, V. & Pascal, V., *Rec. Med. Veter.*, **135** (1959), 709.
14. Faure, J., Guerin, A., Loiseau, P., Dutertre, F., Got, M. & Fressy, J., *Revue de Naurologie*, **100** (1959), 344.
15. Robertson, C. H., Griffin, A. C. & Richardson, H. L., *Cancer Res.*, **13** (1953), 776.
16. Baba, T., *Gann.*, **48** (1957), 145.
17. Lacassagne, A., Buu-Hoï, N. P., Hurst, L. & Giao, N. B., *C. R. Acad. Sci.*, **258** (1964), 5763; Lacassagne, A., Buu-Hoï, N. P., Ferrando, R. & Giao, N. B., *ibid.*, **260** (1965), 287.
18. Sherrington, C., The Integrative Action of the Nervous System, (1947), Cambridge University Press.
19. Snyder, S. S., Axelrod, J., Wurtman, R. J. & Fischer, J. E., *J. Pharmac. exp. Ther.*, **147** (1965), 371.
20. Konstantinova, M. S., *Dokl. Akad. Nauk SSSR*, **165** (1965), 974.
21. Horgan, V. J. & Philpot, J. St. L., *Nature*, **192** (1961), 662.
22. Uskov, F. N., *Dokl. Akad. Nauk SSSR*, **164** (1964), 61.
23. Thompson, R. S., Gautieri, R. E. & Mann, D. E. (Jr), *J. pharm. Sciences*, **54** (1965), 595.